

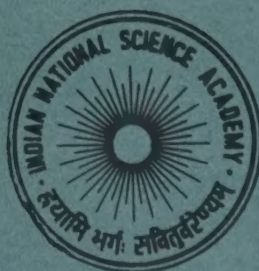
# Profiles in Scientific Research

## Contributions of the Fellows

---

VOLUME 2

---



INDIAN NATIONAL SCIENCE ACADEMY  
NEW DELHI



'Profiles in Scientific Research : Contributions of the Fellows' is a two-volume compilation consisting of articles on the research contributions of contemporary Fellows of the Indian National Science Academy in their own words. A list of chosen important publications of each author is appended. The entries, totalling 441, are arranged subject-wise in the following sequence :

Volume 1 : Mathematical Sciences,  
Physics, Chemical Sciences,  
Engineering & Technology,  
and Earth Sciences

Volume 2 : Plant Sciences, Animal  
Sciences, Medical Sciences,  
Biochemistry and Bio-  
physics, Agricultural, Animal  
Husbandry, Fisheries and  
Forestry).

It is hoped that the publication will serve as an authoritative source-book of scientific achievements of leading Indian scientists. This venture of the Academy is also intended to provide material for the compilation of modern history of science in India.

(Cover design : Gopi Gajwani)

Indian National Science Academy,  
Bahadur Shah Zafar Marg  
New Delhi-110002.











**Profiles in Scientific Research :  
Contributions of the Fellows**







# Profiles in Scientific Research : Contributions of the Fellows

## VOLUME 2

(Plant Sciences, Animal Sciences, Medical Sciences,  
Biochemistry and Biophysics, Agriculture,  
Animal Husbandry, Fisheries and Forestry)

1986



Indian National Science Academy  
Bahadur Shah Zafar Marg  
New Delhi-110002



Progress in Scientific Research:  
Contributions of the Fellows

VOLUME 2

Physical Sciences, Animal Sciences, Medical Sciences,  
Botany and Horticulture, Agriculture,  
Animal Husbandry, Fisheries and Forestry

© Indian National Science Academy, New Delhi

1986

July 1986



Printed and Published by A K Bose, Executive Secretary, Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi-110 002 and printed at Kapoor Art Press, A 38/3, Mayapuri Industrial Area, Phase I, New Delhi-110 064.



## PREFACE

With the rapid strides being made in Science and Technology it is becoming increasingly difficult to have a proper appreciation of the contributions of fellow scientists. Fellows of the Academy would certainly be interested to learn about the research endeavours and accomplishments of the leading scientists in the country. The Academy has been publishing biographical memoirs of deceased Fellows, but there is a greater need for a document providing authentic accounts of the contributions of contemporary Fellows. The Council of the Academy agreed to the suggestion made by me to bring out the volume entitled 'Profiles in Scientific Research : Contributions of the Fellows'. Fellows were requested to write brief accounts of their work and choose 15 representative publications. This had a mixed reaction. Some Fellows were of the view that writing about themselves may be immodest. Others were somewhat surprised but recognised that it was worthwhile to make a statement on their work or on a certain area of science in which they have played a part. The response to our appeal has been overwhelming, and four hundred and forty one Fellows out of the total Fellowship of five hundred and thirty have indeed sent their contributions.

The publication is in two volumes and the contributions are arranged subject-wise. In order to retain the original flavour and style of the Fellows, minimum alterations have been made in the manuscripts. The Profiles would be periodically revised in the future and would become an important source book for research workers and provide material for compiling the history of science in India at a later stage.

The views expressed in these volumes are those of individual Fellows and not of the Academy. A few prominent scientists have not sent their entries. The value of this volume would have been greater if the entire Fellowship had been covered.

I thank all the Fellows who have spared their valuable time in preparing the entries. Special appreciation is extended to Professors S K Joshi and H Y Mohan Ram, Secretaries, for giving a concrete shape to the idea mooted by the Academy and to Shri R N Sharma, former Editor of the Publications and Information Directorate, Council of Scientific & Industrial Research, New Delhi, but for whose able and painstaking assistance, this publication would not have been completed in such a short time. I would like to make a special mention of the dedicated work done by Dr Alok Moitra of the Academy in processing the material and in seeing the publication through the press.

New Delhi  
July 1986

C N R Rao  
President  
Indian National Science Academy





# CONTENTS

Preface

## Sectional Committee—VI : Plant Sciences

Y P Abrol	845
S C Agarwala	847
R D Asana	851
D D Awasthi	855
K S Bhargava	859
K S Bilgrami	862
S S Bir	864
M N Bose	870
V L Chopra	874
S N Das Gupta	878
T V Desikachary	882
H C Gangulee	885
P K Gupta	887
S K Jain	890
B M Johri	893
R N Kapil	899
T N Khoshoo	903
B C Kundu	908
R N Lakhanpal	911
C P Malik	915
P N Mehra	919
R Misra	926
H Y Mohan Ram	929
Y S Murty	935
S C Pandeya	940
D D Pant	945
V Puri	947
M B Raizada	953
V S Rama Das	954
P S Ramakrishnan	958
R P Roy	961
T S Sadasivan	965
S B Saksena	969
Y S R K Sarma	972
S P Sen	976
J J Shah	979
A K Sharma	982
Archana Sharma	986
B N Singh	990



J S Singh	994
S K Sinha	997
H S Sohi	1000
K R Surange	1004
R N Tandon	1007
M J Thirumalachar	1009
B S Trivedi	1014
G S Venkataraman	1017

### Sectional Committee—VII : Animal Sciences

T N Ananthakrishnan	1023
N Balakrishnan Nair	1025
M L Bhatia	1028
C M S Dass	1031
J S Datta Munshi	1034
C J Dominic	1039
G P Dutta	1042
Asok Ghosh	1046
S S Guraya	1049
Ishwar Prakash	1056
V G Jhingran	1058
M S Kanungo	1062
Krishna Swarup	1065
G K Manna	1068
K N Mehrotra	1073
Sivatosh Mookerjee	1078
A S Mukherjee	1081
M K K Pillai	1085
M R N Prasad	1088
L S Ramaswami	1092
S R V Rao	1096
M L Roonwal	1098
Salim Ali	1101
A G Sathyanesan	1104
V C Shah	1107
G P Sharma	1109
V P Sharma	1111
Har Dayal Srivastava	1114
P N Srivastava	1118
U S Srivastava	1120

### Sectional Committee—VIII : Medical Sciences

S C Agarwal	1125
B K Anand	1129
Sachchidananda Banerjee	1134
J V Bhat	1140
S N Chatterjee	1146
S R K Chopra	1150
A B Chowdhury	1153
C R Das Gupta	1156

Darab K Dastur	1161
M G Deo	1164
B N Dhawan	1167
H C Ganguli	1171
T K Ghosh	1175
C Gopalan	1178
N Gopinath	1181
Robert Heilig	1184
K R Laumas	1186
Usha K Luthra	1191
K S Mathur	1195
R K Pal	1198
T Ramkrishnan	1203
V Ramalingaswami	1207
B Ramamurthi	1210
Kamal J Ranadive	1212
A P Ray	1216
C R R M Reddy	1221
B B Roy	1226
A K Saha	1232
J K Sarkar	1235
S C Seal	1239
A B Sen	1245
H G Sen	1247
P K Sen	1251
K N Sharma	1255
A K P Sinha	1257
S Sriramachari	1261
P V Sukhatme	1267
K Sundaram	1272
M Swaminathan	1276
G P Talwar	1279
P N Tandon	1286
M S Valiathan	1291
N H Wadia	1293
P N Wahi	1296

### Sectional Committee—IX : Biochemistry and Biophysics

P R Adiga	1305
N Appaji Rao	1310
B K Bachhawat	1315
John Barnabas	1318
Amar Nath Bhaduri	1323
P M Bhargava	1325
B B Biswas	1329
D P Burma	1332
N N Das Gupta	1337
Asoke G Datta	1341
G C Esh	1344
K Ganapathi	1348
J Ganguly	1352



V Jagannathan	1355
C R Krishna Murti	1357
P K Maitra	1360
N R Moudgal	1362
M C Nath	1365
N K Notani	1371
G Padmanaban	1374
G N Ramachandran	1377
L K Ramachandran	1383
N Ramanathan	1388
V S R Rao	1392
V Sasisekharan	1397
P S Sastry	1401
C SivaRaman	1404
A Sreenivasan	1407
R Srinivasan	1409
Joseph Thomas	1412
C S Vaidyanathan	1416

### **Sectional Committee—X : Agriculture, Animal Husbandry, Fisheries and Forestry**

I P Abrol	1421
Akhtar Husain	1425
I S Bhatia	1428
Bishwajit Choudhury	1432
Rajat De	1435
G B Deodikar	1438
P D Dogra	1442
Madhav Gadgil	1447
B P Ghildyal	1450
K S Gill	1453
B M Gupta	1457
H K Jain	1463
J S Kanwar	1466
S Kedharnath	1470
Abrar M Khan	1475
Gurdev S Khush	1479
K B Lal	1483
G Madhava Reddy	1484
V V Modi	1489
S K Mukherjee	1492
B R Murty	1494
B P Pal	1499
R S Paroda	1501
N Parthasarathy	1507
Prem Narain	1510
S S Prihar	1514
K Ramiah	1518
M S Randhawa	1522

N S Randhawa	1524
N G P Rao	1528
Satya P Raychaudhuri	1533
Syama P Raychaudhuri	1536
D P Sadhu	1540
S V S Shastry	1542
N S Subba Rao	1545
M S Swaminathan	1548
J N S Yadava	1552
<b>Index Volume 2</b>	<b>1557</b>
<b>Cumulative Index :</b>	
<b>Volumes 1 &amp; 2</b>	<b>1559</b>





**Sectional Committee—VI : Plant Sciences**  
***(Structural, Developmental, Functional, Genetical,  
Ecological, Taxonomical and Evolutionary Aspects)***





## Y P Abrol

In an attempt to elucidate the causative factors for dehydration of developing tomato seeds, lying in the succulent placenta, it was reported that movement of water takes place against osmotic gradient and is an active process.

Investigations on cyanide production and its metabolism in a number of cyanophoric plants led to the following conclusions: (i) The amino acid precursors of a number of cyanogenic compounds were established; (ii) a new cyanogenic compound in *Nandina domestica* Thunb was identified; and (iii) it was shown conclusively that cyanogens are metabolically active rather than inert end products.

Studies conducted to elucidate the basis for poor quality of *chapati* made from wheat grains of Mexican dwarfs revealed that these have higher phenolase activity as compared to those of the local tall cultivars. High activity of this enzyme is responsible for discoloration of whole meal dough and *chapaties*. A simple test for screening wheat grains for activity was standardized. At the All India Wheat Research Workers' Workshop, it was recommended for use by wheat breeders. In the developing wheat grain there is no synchrony in the appearance of monphenolase and diphenolase, which suggests that there are two different

enzymes and they are not part of the same enzyme complex.

In a collaborative programme involving crosses between Indian commercial cultivars and Hiproly, a genotype was isolated from Ethiopian germ plasm; three strains with high lysine and high protein contents were identified. In the strain 1098-2, lysine content was 4.02-4.16 g/16 g N.

Detailed investigations on the nitrogen economy of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare*) have shown that (i) a major portion of nitrate is reduced by the leaf blades, (ii) the upper leaf blades have suboptimal nitrate assimilatory activity, which is due to non-availability of  $\text{NO}_3^-$  in the soil at that stage; (iii) the nitrate reductase (NR) activity in the cultivars varies and there is higher potential for  $\text{NO}_3^-$  assimilation in the high NR cultivars as compared to low NR cultivars; and (iv) it is possible to enhance the NR activity by making  $\text{NO}_3^-$  available in the soil at later stages of growth; this increases the reduced nitrogen content in the harvest.

Nitrogen, predominantly in the form of ammonia, is lost from the plant canopy. A series of experiments conducted to elucidate the factors responsible for ammonia accumulation and its loss have yielded the following results: (i) Glycine,

asparagine and allantoic acid feeding results in significant ammonia accumulation, (ii) similar levels of ammonia are present in  $C_3$  and  $C_4$  plants, (iii) considerable amount of nitrogen, possibly in the form of ammonia, is lost during the post-flowering phase from the leaves and developing ears of wheat (*Triticum aestivum* L.), and (iv) at higher soil nitrogen levels and under stress, the ammonia levels are higher in the leaves as compared to the controls.

### Selected Publications

- McIlrath W J, Abrol Y P & Heiligman F, Dehydration of seeds in intact fruits, *Science*, **142** (1963) 1681-82.
- Abrol Y P & Conn E E, Non-occurrence of lotusin in *Lotus arabicus* L., *Nature, Lond*, **206** (1965) 399.
- Abrol Y P & Conn E E, Studies on cyanide metabolism in *Lotus arabicus* L., *Phytochemistry*, **5** (1965) 237-42.
- Abrol Y P, Conn E E & Stoker J, Studies on identification, biosynthesis and metabolism of a cyanogenic glucoside in *Nandina domestica* Thunb, *Phytochemistry*, **5** (1966) 1021-27.
- Blumenthal-Goldschmidt S C, Hendrickson H A, Abrol Y P & Conn E E, Cyanide metabolism in higher plants. IV. The biosynthesis of  $\beta$ -cyanoalanine, *J biol Chem*, **243** (1968) 5302-7.
- Abrol Y P, Uprety D C, Ram A & Tikoo S, Phenol colour reaction as an indicator of Chapati quality in wheat, *Sabao Newslett*, **3** (1971) 17-21.
- Taneja S, Abrol Y P & Sachar R C, Modulation of o-diphenolase and monophenolase enzymes during the development of wheat grains, *Cereal Chem*, **52** (1974) 547-55.
- Abrol Y P, Kaim M S & Nair T V R, Nitrogen assimilation, its mobilization and accumulation in wheat (*Triticum aestivum* L.) grains, *Cereal Res Comm*, **4** (1976) 431-40.
- Nair T V R & Abrol Y P, Studies on nitrate reducing system in developing wheat ears, *Crop Sci*, **17** (1977) 438-42.
- Grover H L, Nair T V R & Abrol Y P, Nitrogen metabolism of the upper three leaf blades of wheat at different soil nitrogen levels. I. Nitrate reductase activity and content of various nitrogenous constituents, *Physiol Planta*, **42** (1978) 287-92.
- Nair T V R, Grover H L & Abrol Y P, Nitrogen metabolism of the upper three leaf blades of wheat at different soil nitrogen levels. II. Protease activity and mobilization of reduced nitrogen, *Physiol Planta*, **42** (1978) 292-300.
- Proc National Symposium on Nitrogen assimilation and crop productivity*, edited by S P Sen, Y P Abrol and S K Sinha (Associated Publishing Co, New Delhi) 1978, pp. 311.
- Naik M S, Abrol Y P, Ramarao C S & Nair T V R, Nitrate assimilation—Its regulation and relationship to reduced nitrogen in higher plants, *Phytochemistry*, **21** (1982) 495-504.
- Abrol Y P, Sawhney S K & Naik M S, Light and dark assimilation of nitrate in plants, *Pl Cell Envir*, **6** (1983) 595-600.
- Abrol Y P, Kumar P A & Nair T V R, Nitrate uptake, assimilation and grain nitrogen accumulation, *Adv Cereal Sci Technol*, **6** (1984) 1-48.
- Singh P, Kumar P A, Abrol Y P & Naik M S, Photorespiratory nitrogen cycle—A critical evaluation, *Physiol Planta*, **66** (1985) 169-76.



## S C Agarwala

Agarwala started plant nutrition work in 1950 at Long Ashton Research Station, Bristol University. On his return to India in 1953, he established a school of plant nutrition at the Botany Department, Lucknow University and since then has been carrying out research on different facets of plant nutrition, plant-soil relationship and soil fertility, with the active collaboration of his students and research associates, his principal associate being Prof. C P Sharma, currently Professor and Head of the Department of Botany, Lucknow University, Lucknow. The other prominent associates were: Prof. N K Mehrotra, Prof. S Farooq, Prof. C Chatterjee, Dr A Kumar, Dr S S Bisht, Dr P N Sharma, Dr S C Mehrotra, Dr B D Nautiyal and Dr(Mrs) N Nautiyal.

The major contributions of Agarwala are discussed below.

(i) It was established for the first time that molybdenum is required in higher plants even when nitrogen is supplied in reduced form.

(ii) 'Whiptail' effects were reproduced in cauliflower grown in sand culture at a molybdenum level (0.00005 ppm) intermediate between that causing severe deficiency symptoms and normal growth<sup>2</sup>; these symptoms were also produced when the source of nitrogen was ammonium, urea and glutamic acid<sup>3</sup>. He also showed

that molybdenum is involved directly or indirectly in the synthesis of ascorbic acid, reduction of triphenyl tetrazolium chloride and pectin formation; when the source of nitrogen was nitrate, in molybdenum deficiency there were large decreases in the amino acids glutamic acid, glutamine, aspartic acid, alanine,  $\beta$ -alanine, glycine, lysine and asparagine and accumulation of arginine<sup>4</sup>.

(iii) Agarwala established that deficiency of molybdenum depresses nucleic acids—DNA and RNA. Apart from the reduction in the activity of nitrate reductase, the activity of some iron enzymes was also depressed and the activity of ribonuclease and acid phosphatase was stimulated in molybdenum deficiency<sup>5</sup>.

(iv) At moderate deficiency of molybdenum, copper, zinc, iron and boron, there was retardation in anther, pollen formation and germination of pollen<sup>6</sup>.

(v) Agarwala described the symptoms of deficiency of iron, manganese, copper, zinc, boron and molybdenum and determined the deficiency, sufficiency and toxicity values in a large number of Indian crop plants, e.g. wheat, rice, maize, barley, sorghum, green gram, gram, black gram, mustard, groundnut, sesamum, soybean, sugarbeet and sunflower. Many of the

---

Professor, Botany Department, University of Lucknow, Lucknow-226007; Residence : 79, Badshabagh, Lucknow.

symptoms have been recorded for the first time and the critical values of deficiency, sufficiency and toxicity of these elements in different crops have also been determined for the first time; this information will be of great value in diagnosing deficiency of micronutrients under field conditions.

(vi) It was shown that copper deficiency depressed the concentration of DNA and RNA and the activities of polyphenol oxidase, cytochrome c oxidase, catalase and aldolase and increased the activities of acid phosphatase, peroxidase and ribonuclease. Infiltration of copper to copper-deficient leaves increased the cytochrome c oxidase and peroxidase activities in the presence of cycloheximide, but that of polyphenol oxidase increased in the absence of the inhibitor.

(vii) Excess supply of heavy metals was found to produce iron deficiency symptoms as well as specific effects of these metals. It was further shown that iron enzymes were depressed and  $^{59}\text{Fe}$  was less in plants suffering from heavy metal toxicity<sup>8</sup>.

(viii) It was found that in most plant species, iron supply was not related to tissue iron concentration of leaves<sup>9</sup>, that tissue iron extracted in 1N HCl, 1N oxalic acid, 0.1N EDTA, 1N citric acid and 0.1N DTPA was related to iron supply<sup>11</sup>.

(ix) It was found that rice plants do not grow normally and show induced iron deficiency in sand or solution culture with nitrate nitrogen and iron as ferric citrate or Fe-EDDHA, in which most of the plants grow normally<sup>12</sup>. The iron requirement of rice with nitrate nitrogen is 28 ppm instead of 5.6 ppm.

(x) For the first time, symptoms of acute zinc deficiency and molybdenum deficiency have been reported in sugarcane<sup>13</sup>.

(xi) Two isoenzymes of aldolase in the deficiencies of zinc, copper, manganese and iron were noted, indicating that these micronutrients are involved in maintaining the integrity of the enzyme<sup>12</sup>.

(xii) In barley, plants grown in sand culture at low levels of iron contained 2-5 times as much copper, molybdenum, manganese and zinc as plants supplied iron as high as 25 times.

(xiii) Study of micronutrient requirement of high yielding varieties of Indian crop plants has revealed marked differences in susceptibility to deficiency of iron, zinc, manganese, copper, molybdenum and boron.

(xiv) Rice variety sabarmati was found suitable for low zinc soils and gram variety T1 was found suitable for soils prone to iron deficiency. In gram, varietal susceptibility to iron chlorosis has been found to be related to genotypic differences in uptake and translocation of iron.

(xv) For the first time, the symptoms of iron, manganese, copper, zinc, molybdenum and boron deficiencies in some fruit trees, e.g. papaya, guava and mango, have been reported<sup>14</sup>. In addition to reporting the values of deficiency and sufficiency of each of the above-mentioned elements, some metabolic aspects have also been worked out.

(xvi) Total and available micronutrients, iron, manganese, copper, zinc, molybdenum and boron, in six representative profiles drawn from each of



the principal soil types of Uttar Pradesh, have been worked out. Alluvial soils were found to be low in total and available molybdenum and available boron in semi-desert soils, available copper in most alluvial and tarai soils. Available molybdenum and copper in many alluvial soils are lowest among those reported for other world soils.

(xvii) Micronutrient concentrations in usar soils (saline and alkaline) have been worked out and an attempt has been made to resolve the complex effect of alkalinity and salinity.

In artificially alkalized soils, symptoms of zinc, copper and iron could be induced. Boron deficiency symptoms appeared when salinity was raised.

(xviii) A method for determining total and available molybdenum in soils has been standardized.

(xix) Iron requirement of *Aspergillus sulphureus* was found to be 2 ppm as against 0.2 ppm for *A. flavus*, *A. terreus* and *A. niger*; the latter was found to have the highest molybdenum requirement (1 ppb Mo), whereas it was 0.2 ppb for the other three *Aspergillus* species mentioned above; deficiency of iron and molybdenum reduced the dry weight spore formation and affected the specific activities of several enzymes.

Addition of cycloheximide along with iron decreased the specific activity of succinic dehydrogenase, aconitase and nitrate reductase in *Aspergillus* and not that of catalase and peroxidase, which were all restored on the addition of iron<sup>15</sup>.

On addition of molybdenum to molybdenum deficient *A. niger* felt only the specific activity of nitrate reductase

recovered and not those of catalase, peroxidase and succinic dehydrogenase in the presence of cycloheximide, which were depressed in molybdenum deficiency<sup>15</sup>.

Agarwala has co-authored two books<sup>16,17</sup>

### Selected Publications

1. Agarwala, S C, Relation of nitrogen supply to the molybdenum requirement of cauliflower grown in sand culture, *Nature, Lond*, **169** (1952) 1099.
2. Hewitt E J & Agarwala S C, Production of 'whiptail' in cauliflower grown in sand culture, *Nature, Lond*, **167** (1951) 732.
3. Agarwala S C & Hewitt E J, Molybdenum as a plant nutrient. VI. The effect of molybdenum supply on the growth and composition of cauliflower plants given different sources of nitrogen supply in sand culture, *J hort Sci*, **30** (1955) 163-80.
4. Hewitt E J, Agarwala S C & Williams A H, Molybdenum as a plant nutrient. VIII. The effect of different molybdenum levels and nitrogen supplies on the nitrogen fractions in cauliflower plants grown in sand culture, *J hort Sci*, **32** (1957) 34-48.
5. Chatterjee C, Nautiyal N & Agarwala S C, Metabolic changes in mustard plants associated with molybdenum deficiency, *New Phytol*, **100** (1985) 511-18.
6. Agarwala S C, Chatterjee C, Sharma P N, Sharma C P & Nautiyal N, Pollen development in maize plants subjected to molybdenum deficiency, *Can J Bot*, **57** (1979) 1946-50.
7. Agarwala S C, Sharma P N, Chatterjee C & Sharma C P, Development and enzymatic changes during pollen development in boron deficient maize plants, *J Pl Nutr*, **3** (1981) 337-44.
8. Agarwala S C, Bisht S S & Sharma C P, Relative effectiveness of certain heavy metals in producing toxicity and symptoms of iron deficiency in barley, *Can J Bot*, **55** (1977) 1299-1307.
9. Agarwala S C & Sharma C P, The relationship of iron supply to the tissue concentration of iron, chlorophyll content and catalase activity of barley plants grown in sand culture, *Physiol Plant*, **14** (1961) 275-83.
10. Agarwala S C, Sharma C P & Farooq S, Effect of iron supply on growth, chlorophyll, tissue iron

- and activity of certain enzymes in maize and radish, *Pl Physiol*, **40** (1965) 493-99.
11. Mehrotra S C, Sharma C P & Agarwala S C, A search for extractants to evaluate the iron status of plants, *Soil Sci Pl Nutr*, **31** (1984) 155-62.
  12. Agarwala S C, Bisht S S, Sharma C P & Afzal A, Effect of deficiency of certain micronutrients on the activity of aldolase in radish plants grown in sand culture, *Can J Bot*, **54** (1976) 76-78.
  13. Agarwala S C, Chatterjee C, Nautiyal B D, Dube B K & Nautiyal N, Induction of deficiency of zinc, copper and molybdenum in sugarcane and its effect on growth, sugar concentration and some enzyme activities, *Sugarcane*, **6** (1985) 1-7.
  14. Agarwala S C, Nautiyal B D & Chatterjee C, Manganese, copper and molybdenum nutrition of papaya, *J hort Sci*, in press.
  15. Agarwala S C, Nautiyal N & Chatterjee C, Iron nutrition of four *Aspergillus* species, *Trans Br mycol Soc*, in press.
  16. Agarwala S C & Sharma C P, *Recognising micronutrient disorders of crop plants on the basis of visible symptoms and plant analysis* (Botany Department, Lucknow University, Lucknow), 1979.
  17. Agarwala S C & Sharma C P, *Micronutrients in agriculture* (Botany Department, Lucknow University, Lucknow) 1979.

## R D Asana

The major research findings of Asana prior to his joining the Botany Division of the Indian Agricultural Research Institute (IARI), New Delhi, in 1946, were as follows: (1) Plane-polarized light was found not to influence photosynthesis, (2) The first negative phototropic curvature of the *Avena coleoptile* was associated with relatively larger concentration of auxin and growth on the lighted side, (3) The rice plant (*Oryza sativa* L) assimilated  $\text{NO}_3\text{-N}$  as effectively as  $\text{NH}_4\text{-N}$ , provided  $\text{pH}$  was maintained around 6, (4) Seasonal variation in the yield of rain-fed sugarcane, in north-Bihar, was largely mediated through nitrogen uptake, as influenced by rainfall and soil conditions.

The main areas of work of Asana at IARI related to (i) raising more than one generation of wheat in a year; (ii) effect of sound and electric current treatment of seed on plant growth; (iii) resistance of wheat to soil salinity; (iv) lodging resistance of tall wheats; (v) effects of microelement deficiencies and growth regulators on crop growth and weeds; and (vi) physiological analysis of the yields of wheat, rice, sorghum and induced tetraploid of *toria*.

Some salient results of studies pertaining to area (vi) are discussed below in brief.

### Analysis of Yield under Water Stress

The tillering potential of wheat genotypes was not fully expressed under *barani* (rainfed) cultivation and the main-shoot ears largely contributed to grain yield. Grain number per ear and 1000-grain weight had more potent influence (beta values) than ear number on yield under moisture stress.

Other *barani* trials conducted with tall and semidwarf cultivars (earring time ranging from 60 to 100 days) showed (i) an association between ear number and yield per unit area with November to January rainfall and little consistent effect of February-March rainfall; (ii) ear number per unit area barely exceeded plant number; (iii) the tall NPs and semi-dwarf 'Kalyan sona', earing in 90-100 days from sowing, succeeded better than early varieties because of more root growth in, and better extraction of soil moisture from, lower depths.

Grain development in wheat was studied under better controlled water stress in pot culture.

Grain-floret ratio decreased with increase in floret number, but grain number depended upon the floret number of a genotype. The pattern of distribution of grains, dry matter, nutrients and water along the ear axis was not altered by water



stress. At "permanent wilting" of leaves, the water content of stem, leaves and spikelets was reduced by 15, 40 and 5% respectively. Water stress after anthesis reduced grain number and protein-total nitrogen ratio and increased the dry matter in the ear. Grain growth continued at the same rate as in the control for 3-4 weeks in spite of rapid yellowing of the shoot. Grain filling was obviously maintained by stem-sugars, which decreased considerably. Thereafter, grain growth declined rapidly due to desiccation. The ultimate lower grain weight was due to starch content.

### **Effect of Temperature Stress**

Rise in temperature during grain development limits grain yield under Indian conditions. Under controlled environment (in Australia), 16% reduction in yield occurred for 6°C rise in temperature between day temperature limits (25-31°C) and night temperatures (9-12°C) 12 days after anthesis to maturity, in five cultivars of wheat. The cultivars responded similarly.

### **Analysis of Yield of Monsoon Sorghum**

At Delhi, sorghum hybrid 'CSH-2' did not express its high yield potential (as compared with 'CHS-1') consistently because of its late flowering. Its panicle emergence was inhibited under increasing soil moisture stress when rainfall ceased early.

### **Analysis of Yield under Adequate Nutrient and Water Supply**

This study was carried out mostly under pot culture where lodging did not occur. In several tall cultivars, grain weight per ear increased at a similar rate during the first 3-4 weeks from anthesis. Since there was a

similar increase in the dry matter of the whole shoot, photosynthetic activity during this stage probably did not vary with cultivar. Variation in grain weight appeared thereafter. For example, 'NP 824', with larger spikelet and grain number per ear, and slower rate of shoot senescence, eventually outyielded 'NP 823'. In spite of the inconsistent difference in the rate of shoot senescence, 'Pb C 281', with lower grain number per ear, produced higher grain weight per ear than 'NP 720'. Did photosynthetic activity vary during this period? The effect of variation in light intensity was investigated in this connection.

During the first fortnight from anthesis, grain weight per ear in these cultivars increased at a declining rate under light intensities below sunlight. Under the lowest light intensity (260 FC), grain weight increased to the level of 45% of that in sunlight, although the shoot weight did not increase (under 260 FC). Evidently, reserve material in the stem (which decreased in weight in contrast with increase under sunlight) compensated grain growth under 260 FC.

During the fortnight before maturity, the shoot weight did not increase and remained constant under light intensities from 230 FC to sunlight. The stem weight decreased substantially and grain weight increased uniformly under all light intensities. During this period, about 20% of the final grain weight was added. In view of the constancy of the shoot weight, and equal increases in grain weight under various light intensities, it appears that 'sink activity', rather than photosynthetic activity, was involved in the eventual variation in grain yield ('NP 824' > 'NP 823', 'Pb C 281' > 'NP 720').

Under exposure to various light intensities, throughout from anthesis to maturity, 'NP 824' suffered more than 'NP 823' under low light intensities. Brief exposures for about a week to low light intensities, however, showed that grain growth in 'NP 824' suffered more only during 15-22 days from anthesis. It is not clear why photosynthetic rate should be depressed more in 'NP 824' only during this stage. Perhaps the developmental processes in the endosperm were more sensitive to light intensity in 'NP 824'.

Among the tall weak-strawed NPs, 823, 824 and 976, lodging-resistant semi-dwarf 'Senore 64' and 'Kalyan sona' and dwarf hybrids, 'HDs 1949' and '1955', 'Kalyan sona', with high grain number per ear and grain growth rate, large 'flag leaf area duration' (large 'flag leaf area') and peduncle diameter (phloem area?) was the highest yielder. The performance of 'HD 1949', with a strikingly high ear number, was limited by low values of the attributes characteristic of 'Kalyan sona'. Under light intensities below sunlight from anthesis to maturity, 'Kalyan sona' maintained its superiority in yield over the tall and dwarf cultivars.

### Concluding Remarks

The performance of *barani* wheat and monsoon sorghum depended largely upon flowering time, root depth, grain number per ear and grain growth rate, as determined by temperature and day-length regimes of a location. On the basis of these considerations, an ideotype of *barani* wheat was formulated and suggestions were offered for improvement of monsoon sorghum. It is, however, felt that special physiological and morphological attributes can only

meagrely substitute for adequate water supply.

Under optimal nutrient and water supply, sink activity, besides photosynthesis, determined genotype variation in yield of tall wheats. Among the tall, semidwarf and dwarf cultivars tested, 'Kalyan sona' was the highest yielder by virtue of the parameters already noted. It remains to be investigated whether 'Kalyan sona' has more efficient photosynthesis and transport system besides high sink activity. It might also be useful to investigate if a more efficient type than 'Kalyan sona' could be developed by combining the high ear potential of 'HD 1049' with the other useful attributes of 'Kalyan sona'. It is also to be established as to what determines spikelet number and floret fertility.

In the end, the author expresses his thanks to numerous post-graduate students and colleagues for their collaboration and active participation in these investigations.

### Selected Publications

1. Asana R D, On the relation between the distribution of auxin in the tip of the *Avena coleoptile* and the first negative phototropic curvature, *Ann Bot*, **2** (1938) 955-57
2. Asana R D, Growth analysis of the sugarcane crop in north Bihar (India). I, Seasonal variation in growth and yield of unmanured plots, *Ann Bot*, **14** (1950) 465-86.
3. Asana R D, The problem of assessment of drought resistance in crop plants, *Indian J Genet Pl Breed*, **17** (1957) 570-78.
4. Asana R D, Analysis of drought resistance in wheat, *Arid Zone Res*, **16** (1961) 183-90.
5. Asana R D, Measurement of drought resistance in crop plants, *Arid Zone Res*, **25** (1965) 415-19.
6. Asana R D & Williams R F, The effect of temperature stress on grain development in wheat, *Aust J agric Res*, **16** (1965) 1-13.

7. Asana R D, In quest of yield, *Indian J Pl Physiol*, **11** (1968) 1-10.
8. Asana R D, Growth habits of crops of nonirrigated areas, Important characteristics of plant types, *Indian Fmg*, **18** (1968) 25-27.
9. Asana R D & Sarin M N, Crop physiology in India, *ICAR Tech Bull (Agric Series)* No 16 (1968) 1-98.
10. Asana R D, I tinker with crop physiology, *Indian J Pl Physiol*, **19** (1976) 1-7.
11. Asana R D, Physiological approaches to breeding of drought-resistant crops, *ICAR Tech Bull (Agric)*, No 52 (1976) 1-48.
12. Asana R D, Effect of winter rainfall on nonirrigated wheat, *Proc Fifth International Wheat Genetics Symposium*, Vol 2 (Indian Soc Genet & Pl Breed, New Delhi) 1978, 907-15.
13. Asana R D, What is wrong with crop production under rain-fed cultivation? In *Plant physiology: An underutilized potential for increasing crop productivity*, *Proc Seminar*, Food & Agriculture Committee, Dept Atomic Energy, Govt of India, Bangalore, March 1981, 151-72.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.



## D D Awasthi

Taxonomic studies on Indian lichens had not been taken up in India prior to the middle of the present century, except for a single publication of Chopra. Most of the type and other collections of lichens from India were described by the European lichenologists and thus the specimens were also lodged in different European herbaria, though a few duplicates of Indian lichens and certain foreign lichens had been preserved in the Calcutta herbarium. The paucity of lichen collections from India even at Calcutta herbarium, as examined by me in late forties, prompted me to take up the challenge to develop a lichen herbarium and to carry out taxonomic investigations on them in India itself. Representative collections from different parts of India, particularly from the lichen-rich regions of the Himalayas and the hilly regions of southern India, were made. The foremost detailed paper published in 1957 dealt with 7 new species—two of *Cetraria*, two of *Physcia*, two of *Anaptychia* and one of *Peccania*. In another publication in the same year, a new species of *Parmelia* from Kodaikanal was reported.

Since several specimens of *Physcia* and *Anaptychia* had accumulated and requisite literature was available, the 13 species of *Physcia* and 14 species of *Anaptychia* from India were described in detail in 1960. Simultaneously, a lichen collection from the region of Cho-Oyu in East Nepal was

also made available to me for study; it comprised 38 species, of which five were new reports for the Himalayas. Another publication in 1961 dealt with the macro-lichens mostly from Arunachal Pradesh and comprised 72 taxa.

During the course of an advanced training on taxonomy of lichens at the Colorado University, USA, I specially studied certain micro-lichens. As many as 43 species and three genera were found to be new reports from India and Nepal in the paper published in 1963.

Inadequacy of consolidated information on Indian lichens was met by me by bringing out a compilation in 1965<sup>1</sup>, which included a historical sketch of lichenological progress on Indian lichens since 1753, and gave a list of about 1300 taxa of lichens reported till 1965 from the Indian subcontinent (India, Nepal, Pakistan and Ceylon). This catalogue formed the base for further work on Indian lichens and has, therefore, often been referred to by foreign lichenologists whenever a reference to Indian lichen taxa is needed.

Research projects financed by the Council of Scientific and Industrial Research, New Delhi, University Grants Commission, New Delhi, and the State Council of Science and Technology, Lucknow, made it possible for me to make further comprehensive collections of

---

Emeritus Scientist (CSIR), Department of Botany, Lucknow University, Lucknow-226007; Residence : 38, Khurshed Bagh, Lucknow-226007.

lichens from Darjeeling district in east Himalayas, several areas from Kumaun to Kashmir in western Himalayas and Nilgiri and Palni hills in southern India. During the course of the projects and subsequently, there had been good progress in investigations on Indian lichens by me and the research fellows associated with the projects. Several notes dealing with new taxa, new combinations and new reports were published. New species, one each of *Mycoblastus*, *Tomasellia*, *Phlyctella* and *Placopsis*, along with interesting observations on two species of *Haematomma*, were published in 1968 in joint authorship with M R Agarwal. The same authors described seven species of *Cryptothecia* from Darjeeling district in 1969; this was followed by an enumeration of the lichen taxa occurring in the tropical and subtropical regions of Darjeeling district.

Investigations on specimens of *Alectoria* from Darjeeling and Nepal revealed that an earlier described taxon, *A. acanthodes* Hue, was to be considered a synonym, while a new species, *A. confusa* Awas., needed to be established for certain widely distributed *Alectoria* specimens. The paper was published by me in 1970.

Collections of lichens from Nilgiri and Palni hills were found to be extremely rich in lichen taxa and several papers in joint authorship with K P Singh were published. These included a new species of *Stirtenia*, two species of *Microglæna*, two species of *Porina*, a few new combinations, and a study of the foliicolous lichens of Nilgiri and Palni hills<sup>2</sup>, in which detailed descriptions of 42 species under 13 genera with keys for their identification were provided.

Recognition of *Heterodermia* as a genus by most of the lichenologists prompted me to publish in 1973 as many as 23 new combinations of its Indian taxa.

Investigations on Graphidaceous lichen taxa were published in joint authorship with K P Singh in several papers. They included revision of the descriptions of several taxa, detailed account of 11 species of genus *Phaeographina* in 1973, a few new taxa and several new reports from Palni and Nilgiri hills, reinvestigations on certain taxa of *Graphis*, and finally a monographic revision of the 28 Indian species of *Phaeographis* in 1981.

A world monograph of the genus *Dirinaria* based on borrowed specimens from 35 herbaria from the world, and comprising 26 species was published by me in 1975<sup>3</sup>. Four new species and 10 new combinations were described in that publication. Lichens collected by me from the region of Pindari Glacier and on route were found to comprise 122 species; of these, 12 species were new reports. In another publication<sup>4</sup>, the large macro-lichen genus *Parmelia* was treated in detail in respect of 102 species from India, of which 5 species were new to science.

The Indian taxa of the genus *Leptogium* were found to comprise 37 species<sup>5,6</sup>, which included two new species.

In the meantime, an interesting new lichen genus, *Heppsora*, with a single species, *H. indica* Awas. & K Singh, was described on a material collected from Palni hills.

Publications in joint authorship with S R Singh included a detailed work on the 11 species of *Rhizocarpon* from India. In an enumeration and description of 74 taxa of lichens collected in 1976 from the region of



Gangotri and Gomukh and published in 1978, two new species, one each of *Physcia* and *Anaptychia* and 14 new reports, were recorded. This was followed by a publication on lichens of Mt Abu, in Rajasthan<sup>7</sup>, in which 32 lichen taxa were reported; these included five new species, one each of *Heppia*, *Phylliscum*, *Physcia*, *Thyrea* and *Zahlbrucknerella*.

In connection with the typification of *Helminthocarpon*<sup>8</sup>, it was found that the genus comprises two species only; a new genus *Cyclographina* was created to include certain other taxa.

The genus *Collema* was found to comprise 32 species from India<sup>9</sup>. All the species were keyed out and described in detail. A note by Awasthi and Upreti dealt with the 22 species of lichens growing in the Lalbagh garden of Bangalore. In another publication, the same authors described two new species, one each of *Buellia* and *Lopadium*. Typification of *Rocella montagnei* led to the recognition of a new species of the genus by me in 1981. In a detailed account of the micro-lichen genus *Buellia*, 39 species were dealt with, 12 species of which were new to science<sup>10</sup>. In two other publications<sup>11,12</sup>, 14 species of *Peltigera* and the family Stictaceae (genera *Lobaria*, *Pseudocyphellaria* and *Sticta*) respectively were treated in detail. In the latter, 11 species of *Lobaria*, 6 species of *Pseudocyphellaria* and 12 species of *Sticta* were described. In a similar monographic work on genus *Pyxine*<sup>13</sup>, 21 species were dealt with, of which 6 taxa were new. In another publication, 24 species of *Cetraria* from India and Nepal<sup>14</sup> were described in detail. One species was new to science, and three new combinations were made. A detailed account of the taxa of genera *Alectoria* (1

species), *Bryoria* (14 species) and *Sulcaria* (2 species) occurring in India and Nepal was published in 1985 by D D Awasthi and G Awasthi. Two new species of *Bryoria* were described.

Thus, a majority of the macro-lichen genera in India have been dealt with exhaustively. The accounts comprise keys to the identification of the species, their detailed morphological description with photographs and notes on distribution and interrelationship of the closely allied taxa. The progress made during the past 20 years has been acclaimed nationally and internationally. To date, two new genera (one from India), over 75 new species, 15 new varieties, 50 new combinations and over 160 new reports of lichen taxa from India have been published.

In spite of active and continuous progress made with the facilities available, much remains to be done for the publication of a 'Lichen Flora of India'—the final goal. Several micro-lichen genera still remain to be investigated in the Indian context. Some of them are under investigation under my supervision as Emeritus Scientist of C.S.I.R., New Delhi. Some work is also going on in three other centres in India. The present position, prospects and problems in respect of Indian lichenology have been discussed in detail elsewhere<sup>15</sup>. Establishment of a Lichenology Research Centre at Botany Department, Lucknow vis-à-vis increase in the number of research personnel and a corresponding increase in research facilities seem to be the prime need if we want to have the *Lichen Flora of India* in the near future.



### Selected Publications

1. Awasthi D D, Catalogue of lichens from India, Nepal, Pakistan and Ceylon, *Beih Nova Hedw*, **17** (1965) pp. 137.
2. Awasthi D D & Singh K P, A synopsis of the foliicolous lichens from Nilgiri and Palni hills, India, *Geophytology*, **3** (1973) 13-23.
3. Awasthi D D, A monograph of lichen genus *Dirinaria*, *Biblioth Lichenol*, **2** (1975) pp. 108.
4. Awasthi D D, Lichen genus *Parmelia* in India, *Biol Mem*, **1** (1976) 155-229.
5. Awasthi D D & Akhtar P, The genus *Leptogium* (Sect *Mallotium*) in India, *Norw J Bot*, **24** (1977) 59-71.
6. Awasthi D D & Akhtar P, The lichen genus *Leptogium* (Sects *Leptogium*, *Leptogiosis* and *Homodium*) in India, *Geophytology*, **8** (1979) 189-204.
7. Awasthi D D & Singh S R, New or otherwise interesting lichens from Mt Abu, Rajasthan, India, *Norw J Bot*, **26** (1979) 91-97.
8. Awasthi D D & Joshi M, The lichen genera *Helminthocarpon*, *Cyclographa* and *Cyclographina* (gen nov), *Norw J Bot*, **26** (1979) 165-77.
9. Akhtar P & Awasthi D D, The lichen genus *Collema* in India, *Biol Mem*, **5** (1980) 13-29.
10. Singh S R & Awasthi D D, Lichen genus *Buellia* in India, *Biol Mem*, **6** (1981) 169-96.
11. Awasthi D D & Joshi M, Lichen genus *Peltigera* from India and Nepal, *Kavaka*, **10** (1982) 47-62.
12. Joshi M & Awasthi D D, Lichen family *Stictaceae* in India and Nepal, *Biol Mem*, **7** (1982) 165-90.
13. Awasthi D D, *Pyxine* in India, *Phytomorphology*, **30** (1982) 359-79.
14. Awasthi D D, Lichen genus *Cetraria* in India and Nepal, *Bull Bot Surv India*, **24** (1983) 1-27.
15. Awasthi D D, Present position, prospects, and problems of lichenology in India, *Kavaka*, **11** (1983) 1-10.

## K S Bhargava

### Mycology

Studies made on the nutrition of Saprolegniaceae showed that utilization of polysaccharides was correlated with the capacity of the organisms to hydrolyze them. It was also shown that they were able to synthesize their own thiamine from the ingredients of the nutrient solution and some of the thiamine thus manufactured was given off by the mycelium into the solution.

From a study on *Phytophthora phaseoli*, it was shown that the fungus suffers from thiamine deficiency; ammonium ion was found to be toxic for the growth of the fungus, a very unusual finding.

Cytological evidence was obtained that fertilization took place in *Isoachlya anisospora* var. *indica* by the fusion of male and female nuclei, dispelling the view of some of the earlier workers that antheridia, though present, were functionless. It was further shown that only one nuclear division each was present in the oogonium and antheridium and it occurred simultaneously in both.

Studies on the origin and development of the vacuolar system in the vegetative mycelium of four genera of Saprolegniaceae showed that the vacuole arose *de novo* at the tips of young hyphae and also at the tips of germinating zoospores. The

small vacuoles fused to form a network or chain of round or ellipsoidal vacuoles, which, in turn, fused again to give rise to a continuous vacuolar canal. It was shown that the genera belonging to Saprolegniaceae behaved differently from other fungi in respect of accumulation of neutral red in the vacuoles intravitaly, and that vital coloration was possible only in the vicinity of neutrality.

A study on the formation and liberation of zoospores in *Isoachlya* and *Thraustotheca* established that in the so-called homogeneous stage, the plasma membrane bursts and a part of the cell sap is expelled, leading to shrinkage of sporangium.

Mitochondria in the vegetative mycelium of four genera of Saprolegniaceae were seen as long filamentous and slender bodies of varying lengths, mostly parallel to the longitudinal axis and constantly moving under the influence of cytoplasmic currents. At the tips they were granular in shape and were seen in the process of elongation just behind it.

Studies on the mitochondrial system in spore formation in *Isoachlya* and *Thraustotheca* showed that in the young sporangium initial mitochondria were granular or rod shaped. Such forms are present in all the later stages, leading to

---

Formerly, Professor of Botany, Gorakhpur University; Residence . C-16, Nirala Nagar, Opp. P.W.D. Flats, Lucknow-226007.

sporangiospores and cystospores. In the hyphae formed by the germination of spores, granular mitochondria at the tips were followed by rod shaped and filamentous forms.

Ecological studies on aquatic fungi in temperate and subtropical lakes situated at different altitudes in the same locality in Kumaon showed varying distribution of 26 species of Blastocladales, Saprolegniales, Lagenidiales and Peronosporales; seasonal periodicity of the fungi was found to be governed by temperature.

Studies on fungi parasitic to freshwater fishes, Indian Aspergilli, Indian Cercosporae, storage fungi of condiments, market diseases of fruits and vegetables, verticillium wilt in potato tubers and other soil fungi were carried out in our laboratory by me and my associates.

### Plant Pathology and Virology

*Cucurbit virus diseases* : The properties of different strains of cucumber mosaic virus were studied and it was shown that they differed in their host range, symptoms and virulence towards different plants, in their transmissibility by aphids and the physical properties. Inhibitors of infectivity were shown to be present in sugarbeet and *Phytolacca* sp., which made mechanical transmission from these hosts to other hosts difficult.

Several cucurbit viruses, viz., pumpkin yellow mosaic virus, 'pumpkin mosaic virus, cucumis virus 3, strains of cucumber mosaic virus and strains of watermelon mosaic virus were identified in Uttar Pradesh. Detailed studies on different strains of watermelon mosaic were made and several alternative hosts of this virus were identified, leading to the control of

this virus. Leaf extract of *Portulaca grandiflora* was shown to have a powerful inhibitory effect on watermelon mosaic virus.

*Fruit tree virus diseases* : Several virus diseases of fruit trees, viz., plum line pattern, apple mosaic, necrotic leaf spot in peach, peach mosaic, pear mosaic and almond mosaic, were shown to exist in the hills of Kumaon for the first time; heat therapy was found to be effective in the control of plum line pattern and apple mosaic.

*Sugarcane virus diseases* : The presence of A, B, D and F strains of sugarcane mosaic virus was reported from eastern Uttar Pradesh and *Pennisetum purpureum*, *P. typhoides*, *Sorghum vulgare*, *Eleusine indica*, and some other plants were shown to be harbouring this virus, thus acting as alternative hosts for it. The presence and activity of *Aphis gossypii*, *Melanaphis sacchari*, *Lipaphis pseudobrassicae*, *Myzus persicae*, *Rhopalosiphum maidis*, *R. rufiabdominalis* and *Aphis nerii* colonising sugarcane and other plants growing in the vicinity of sugarcane plantations and acting as vectors of this virus during growing periods were correlated with the incidence and transmission of sugarcane mosaic virus in nature.

The presence of mycoplasma-like bodies, possibly the cause of grassy shoot disease of sugarcane, was also shown.

*Papaya virus diseases* : Papaya mild mosaic, distortion ringspot and ringspot isolate of papaya viruses were studied in detail and it was shown that *Myzus persicae* transmitted all the three viruses, *Aphis craccivora* and *A. gossypii* transmitted papaya mosaic and *A. nerii*



and *Lipaphis pseudobrassicae* transmitted papaya ringspot virus only. Papaya mosaic virus was found to be stylet borne. Infectivity of these isolates was inhibited by extracts of *Achyranthes aspera*, *Aloe barbados*, *Capsicum frutescence* and *Carica papaya*. Papaya mosaic was controlled by oil sprays.

Induced apocarp and double papaya formation have been found on papaya plants infected with papaya distortion ringspot virus.

**Virus diseases of ornamentals and other plants:** Virus diseases of several ornamentals, including *Mirabilis jalapa*, *Tropaeolum majus*, petunia, stock, candytuft, sweet rocket, rose, radish, tree tomato, brinjal and *Cynodon* have been studied.

**Inhibition of virus activity:** In earlier studies, *Phytolacca decandra* and *Portulaca grandiflora* were shown to have a powerful inhibitory effect on cucurbit viruses. More recently, virus inhibitors were isolated from leaf extracts of a fern, *Ampelopteris prolifera*, and partially characterized; they were found to be active against cucumber mosaic virus and tobacco mosaic virus. In addition, the leaf extract of *Adiantum venustum* was found to possess inhibitory activity. So far, no such antiviral activity from the leaves of ferns has been reported.

## Selected Publications

1. Bhargava B, Bhargava K S & Joshi R D, Perpetuation of watermelon mosaic virus in Eastern Uttar Pradesh, *Pl Dis Repr*, **59** (1975) 634-36.
2. Bhargava K S, Physiological studies of some members of the family Saprolegniaceae. IV. Carbohydrate requirements, *Lloydia*, **8** (1945) 60-68.
3. Bhargava K S, Physiological studies of some members of the family Saprolegniaceae. V. Growth substances, *Lloydia*, **9** (1946) 13-23.
4. Bhargava K S, Oogenesis and fertilization in *Isoachlya anisospora* var. *indica*, *Trans Br mycol Soc*, **29** (1946) 101-7.
5. Bhargava K S, Formation and liberation of zoospores of *Isoachlya anisospora* var. *indica*, *Bot Gaz*, **112** (1950) 24-31.
6. Bhargava K S, Cytological studies of some members of the family Saprolegniaceae. I. The Vacuome, *Cytologia*, **16** (1950) 72-83.
7. Bhargava K S, Cytological studies of some members of the family Saprolegniaceae. II. The Chondriome, *Cytologia*, **16** (1950) 84-94.
8. Bhargava K S, Some properties of four strains of cucumber mosaic virus, *Ann appl Biol*, **38** (1951) 377-88.
9. Bhargava K S, Sugarcane mosaic: Retrospect and prospects, *Indian Phytopath*, **28** (1975) 1-11.
10. Bhargava K S & Bist N S, Fruit tree virus disease investigations in Uttar Pradesh, *Tidssk Plant*, **65** (1961) 196-203.
11. Bhargava K S & Hari Kishore, Occurrence of Verticillium in potato tubers in India, *Nature, Lond*, **171** (1953) 800.
12. Bhargava K S & Khurana S M P, Papaya mosaic control by oil sprays, *Phytopath Z*, **64** (1969) 338-49.
13. Bhargava K S & Khurana S M P, Insect transmission of papaya viruses with special reference to papaya mosaic virus, *Z Bak Parasit Infect Hyg*, **124** (1970) 688-96.
14. Bhargava K S, Swarup K & Singh C S, Fungi parasitic on certain fresh water fishes of Gorakhpur, *Indian Biol*, **3** (1971) 65-69.
15. Khurana S M P & Bhargava K S, Induced apocarp and double papaya fruit formation in papaya with distortion ringspot virus infection, *Pl Dis Repr*, **54** (1970) 181-83.
16. Pandey A K & Bhargava K S, Isolation and partial characterization of virus inhibitors from the leaf extract of a fern, *Ampelopteris prolifera* (Retz) Copel, *Indian J Pl Path*, **1** (2) (1983) 193-98.
17. Pandey A K & Bhargava K S, Effect of *Ampelopteris prolifera* leaf extract on the activity of tobacco mosaic and cucumber mosaic viruses, *Indian Phytopath*, **37** (1984) 271-77.
18. Rishi N, Okuda S, Arai K, Doi Y, Yora K & Bhargava K S, Mycoplasma like bodies possibly the cause of Grassy shoot disease of sugarcane in India, *Ann Phytopath Soc Japan*, **39** (1973) 429-31.
19. Saksena R K & Bhargava K S, Nitrogen requirements and vitamin deficiencies of *Phytophthora phaseoli* Thaxter, *Proc Indian Acad Sci*, **13** (1943) 45-51.

## K S Bilgrami

Most of the fungi under natural conditions meet their carbon requirements from complex carbohydrates which are degraded to simpler sugars with the help of extracellular enzymes. My initial work comprised the study of various pathways operative in fungi during the utilization of oligo- and polysaccharides. Previously it was considered to be accomplished solely by a hydrolytic process. On the basis of exhaustive work on carbohydrate metabolism of fungi, we established that the so-called hydrolytic enzymes actually act like group-transferases and bring about transglycosidation. The concept of transglycosidation is a major breakthrough in the understanding of the microbial degradation of complex carbon compounds. We have observed that under both natural and laboratory conditions, generally, a basal carbohydrate serves as the acceptor, leading to the formation of higher oligosaccharides of varied chain lengths. We have demonstrated that the nature and chain length of synthetic transient carbohydrates (oligo- and polysaccharides) depend on the nature of the organism, concentration of carbon in the basic source, rate of its breakdown and rate of utilization of the breakdown products by the consumer organism. On the basis of experimental evidences, we have suggested that transglycosidases are helpful to the microbes in keeping the complex carbohydrates "self-locked" for

gradual use by the consumer organism only.

Study of the chemosynthetic ability of the fungi has been another important area of my study. My main interest has been on secondary fungal metabolites, with special reference to the mycotoxins. Synthesis of a wide range of mycotoxins, including aflatoxins, citrinin and zearalenone, on various food and feed substrates, has been studied. The effect of various environmental and storage conditions on mycotoxin elaboration in cereals, pulses, dry fruits and spices has been examined in depth. The toxic effects of aflatoxins and other mycotoxins on different experimental animals have been demonstrated through histopathological and biochemical studies. Adverse effects on liver, kidney, lungs, sex organs, gall bladder, skin and blood were noted. Toxic degeneration of liver parenchyma and formation of large neoplastic elements with hyperchromatic nuclei in the medullary region of the kidney has been demonstrated in animals. Mycotoxin-induced abnormalities in some selected plants have also been studied.

We have also been successful in preventing aflatoxin elaboration on food commodities with the help of mild phenolics like ferulic acid and O-vanillin as well as many natural plant products. We have shown that identification of toxigenic



and non-toxicogenic strains of *Aspergillus flavus* and *A. parasiticus* was possible on the basis of the amino acid pattern of the hyphae. Some carbon and nitrogen compounds like galactose, lactose, cobalt nitrate and sodium nitrite were found to suppress the synthesis of aflatoxin B<sub>1</sub>.

At present, we are examining the mutagenic effects of mycotoxins on animal and plant systems. We have found aflatoxin B<sub>1</sub> to be a strong mutagen. In the near future, we propose to examine the mutagenic effects of other mycotoxins and shall also concentrate on detoxification of aflatoxins with the help of non-toxic natural and synthetic products. Emphasis will also be laid on the study of the biosynthetic pathways and precursors of aflatoxin B<sub>1</sub>.

### Selected Publications

1. Bilgrami K S, Studies on growth responses of some pathogenic species of *Phyllosticta* in different nitrogen media, *Flora*, **154** (1964) 1-11.
2. Bilgrami K S, Studies of formation and role of synthetic oligosaccharides during the utilization of some complex carbohydrates by some pathogenic species of *Phyllosticta*, *Flora*, **154** (1964) 81-88.
3. Bilgrami K S, The utilization of carbohydrates by the species of *Phyllosticta*, *Proc natn Inst Sci India*, **35** (1967) 79-82.
4. Reddy S M & Bilgrami K S, Oligosaccharide synthesis by species of *Helminthosporium*, *Proc Indian natn Sci Acad*, **39** (1973) 203-20.
5. Bilgrami K S, Misra R S & Sinha K K, Aflatoxin production and loss in caloric value of maize seeds due to *Aspergillus parasiticus*, *Curr Sci*, **48** (1979) 642-43.
6. Bilgrami K S, Misra R S, Sinha K K & Singh Premlata, Prevention of aflatoxin production in liquid and solid substrates through some plant extracts, *Indian J exp Biol*, **17** (1979) 1151.
7. Bilgrami K S, Sinha K K & Singh Premlata, Inhibition of aflatoxin production by ferulic acid on some cereals and oilseeds, *Curr Sci*, **50** (1981) 97-98.
8. Bilgrami K S, Sinha K K & Singh Premlata, Prevention of aflatoxin production on some cereals and oilseeds by O-vanillin, *Curr Sci*, **51** (1982) 138.
9. Bilgrami K S, Sinha K K & Singh Anjana, Chemical changes in dry fruits during aflatoxin elaboration by *Aspergillus flavus* Link ex Fries, *Curr Sci*, **52** (1983) 960-64.
10. Bilgrami K S, Mycotoxins in food, *J Indian bot Soc*, **63** (1984) 109-20.
11. Bilgrami K S & Sinha K K, Mycotoxins in cereals, *Rev Trop Plant Pathol*, **1** (1984) 355-74.
12. Bilgrami K S & Sinha K K, Mycotoxin contamination in food and its control, *Indian Rev Life Sci*, **4** (1984) 19-36.
13. Bilgrami K S & Sinha K K, Pollutants of fungal origin, in *Vistas in plant pathology*, edited by Anupam Varma and J P Verma (Malhotra Pub. House, New Delhi) 1986, 71-81.
14. Bilgrami K S & Sinha K K, Mycotoxin induced abnormalities in plants, in *Front. appl. Microbiol.* edited by K G Mukerji, N C Pathak & V P Singh (Print House, Lucknow) 1986, 349-61.



## S S Bir

The work done by Bir pertains primarily to vascular cryptogams (Pteridophytes) and the flowering plants of the Indian subcontinent, with particular reference to the Himalayas, which constitute an important phytogeographic region of the world.

### (I) Pteridophytes

#### (1) Cytology

Chromosomal studies on about 300 taxa of ferns and fern allies, particularly the Asplenioid-Athyrioid and Polypodioid ferns, constitute a major breakthrough on this front. A large number of species were worked out for the first time. This has helped to study the underlying evolutionary mechanisms in the regional floras of India. It has been amply demonstrated that speciation occurs through hybridization, polyploidy (to a greater extent allopolyploidy but autopolyploidy is also instrumental) and apomixis. Comparative work on the floras of three regions—Himalayas, Central India and South India<sup>1-4</sup>—has led to the following conclusions:

(a) The fern flora of the Himalayas (supporting tropical, subtropical and temperate vegetations) with a high preponderance of diploids is different from that of the rest of the Indian subcontinent. Only about 35% of the Himalayan taxa are

polyploids as compared to 57% of South India, whereas figures are intermediate for Central India.

(b) A large number of species show aneuploid numbers as well as the existence of intraspecific polyploid cytotypes. Accordingly, their taxonomy needs a second look.

(c) *Asplenium unilaterale* complex<sup>5</sup> is extremely interesting in the sense that it features the first report throughout the world of the existence of dibasic hybrids ( $2n = 76, 112$  based on  $x = 36$  and  $40$ ) in the ferns. Here, one can clearly visualize as to how new base numbers are evolved through the formation of new taxa by hybridization and polyploidy.

#### (2) Taxonomy

On the basis of a study of materials in the Indian and foreign herbaria, taxonomic revision of *Microsorium*, *Pleopeltis*, *Lepisorus*, *Polypodium*, *Goniophlebium*, *Pyrrosia*, *Phymatodes*, *Vittaria* and *Cystopteris* has been carried out on all India basis. Nearly 20 new taxa of Indian ferns have been described—*Athyrium mehrae* Bir, *A. himalaicum* Ching ex Mehra & Bir, *A. subtriangulare* (Hook.) Bedd. var. *sikkimensis* Bir, *Cornopteris birii* Ching ex Bir, *Cystopteris sikkimensis* Ching ex Bir, *Asplenium laciniatum* Don var. *acutipinna* Bir, *A. planicaule* Wall.

---

Professor, Department of Botany, Punjabi University, Patiala-147002; Residence : A-6, Punjabi University Campus, Patiala-147002.

var. *obtusa* Bir, *A. crinicaule* Hance var. *sikkimensis* Bir, *A. nidus* Linn. var. *acutifolium* Bir, *Polypodium amoneum* Wall. ex Mett. var. *xerophyticum* Mehra & Bir, *Lepisorus excavatus* (Bory) Ching var. *mortonii* Bir & Trikha, *L. excavatus* var. *himalayensis* Bir & Trikha, *L. amaurolepidus* (Sledge) Bir & Trikha var. *longifolius* Bir & Trikha, *L. kashyapii* (Mehra) Mehra var. *major* Bir & Trikha, *L. kashyapii* var. *minor* Bir & Trikha, *A. paucivenosum* (Ching) Bir forma *minus* Bir, *A. paucivenosum* forma *majus* Bir (= *Ceterachopsis birii* Löve & Löve, *Asplenium birii* (Löve & Löve) Bir, Fraser-Jenkins & Lovis)<sup>6</sup>, *Cystopteris fragilis* forma *granulosa* Bir & Trikha and *C. fragilis* forma *himalayense* Bir & Trikha and *Asplenium punjabense* Bir, Fras-Jenk & Lovis. *Ceterach*<sup>6</sup> and *Ceterachopsis*<sup>6,7</sup> as well as *Neottopteris*<sup>7</sup> and *Asplenidictyum* are treated as subgenera of *Asplenium*.

### (3) Systematics and Ecology

Studies on the ferns and fern allies of various regions like Himalayas (Darjeeling, Sikkim and Simla), Western (Mt. Abu), Central (Pachmarhi) and South India (Kodaikanal) have contributed tremendously towards the compilation of the revised "Pteridophytic Flora" of the country. As a result of intensive surveys, several hitherto unrecorded species have been found for the first time in the Indian region. For the first time, illustrated and comparative accounts of the ecological distribution of Pteridophytes in Himalayas, Central India (Pachmarhi hills) and South India (Kodaikanal) have been given. Pteridophytic Flora of Darjeeling and Sikkim Himalayas<sup>7</sup>, Pteridophytic Flora of Garhwal Himalayas<sup>8</sup> and Polypodiaceous Ferns of India<sup>9</sup> compiled on the basis of

decades of surveys in the Himalayas and the study of herbarium material from national and international herbaria, provide an up-to-date nomenclature of the members and list a large number of species not recorded previously from the regions.

### (4) Morphology, Anatomy and Palynology

(a) Through a study of spores of several hundred Indian ferns, the role of palynology has been well established for the separation of the taxonomically confusing taxa.

(b) Anatomical details of Himalayan members of *Asplenium*, *Ceterach*, *Athyrium*, *Diplazium*, *Cystopteris*, *Diplaziopsis*, *Vittaria*, *Equisetum*, etc. have brought to light the importance of such studies in phylogeny.

(c) Morphological details of dermal appendages and gametophytes have been helpful in drawing inter-relationship among Athyrioid, Asplenioid and Polypodioid ferns.

## (II) Flowering Plants

### (1) Cytology

New chromosome numbers have been reported for nearly 500 taxa.

#### (a) Woody Taxa

Till 1972, chromosomal information on the forest trees and other woody taxa was available for the Himalayas only, and even in this phytogeographically important region, several areas were not worked at all. Comparative data from other regions of the country were completely lacking. Beginning with 1976, as many as 600 taxa of forest trees and their relatives were worked out from Garhwal Himalayas and



Central and South India. Work was also done on the identification of plus trees of forestry importance. A large number of species have been shown to be morphovariants and to contain intraspecific cytological races and B-chromosomes. Based on the results for nearly 400 taxa from Garhwal Himalayas and Central India<sup>10,11</sup>, the following conclusions have been drawn:

(i) The hardwoods have, by and large, small size chromosomes. Multivalent formation is observed in a few diploid and polyploid species. However, within the woody species themselves, both large and small sized chromosome species have been identified.

(ii) There is no relationship between the habit of woody species (trees, shrubs and climbers) and their chromosome size or number.

(iii) Incidence of polyploidy is fairly high in the Garhwal Himalayan taxa (22%) and Central Indian taxa (29.3%).

(iv) In Garhwal Himalaya, there is nearly the same incidence of polyploidy among the taxa from tropical and subtropical as well as temperate zones. On the other hand, in Central India, montane subtropical forests have higher incidence of polyploidy as compared to tropical dry deciduous forests.

(v) The earlier contention of cytological conservatism of woody taxa is no longer tenable; the current belief is that these are in fairly active state of evolution as demonstrated by the higher incidence of polyploidy, hybridity and aneuploid numbers.

#### (b) Indian Legumes

Through meiotic studies on nearly 250

legumes from North and Central India, it has been shown that Mimosaceae is not primitive from cytological point of view, although morphologically the group is considered primitive. Also, there is no cytological evidence that low numbered Caesalpinioid and Papilionoid genera were derived from contemporary elements in Mimosaceae, most of which have high numbers. In the case of Central Indian legumes, Bir and Kumari<sup>12</sup> showed that Papilionaceae possess the lowest number of polyploids, followed by Mimosaceae and the highest incidence is in Caesalpiniaceae. Herbs show lower polyploidy (10.7%) as compared to shrubs (19.5%) and the trees (19.2%). By and large, legumes of north Indian plains show markedly higher incidence of polyploidy as compared to those of the Himalayas and Pachmarhi region (Central India). It is concluded that on the whole, among the Indian legumes, Caesalpiniaceae show the highest incidence of polyploidy (69.57%), (ii) within the tree members, taxa based on lower numbers in Caesalpiniaceae are more adaptable for polyploidy as compared to those of other families with high base numbers, and (iii) occurrence of euploid and aneuploid cytotypes among herbaceous, shrubby and tree species is a common feature, marking out the high evolutionary rate of the legumes.

#### (c) Plants of Economic Importance (Acanthaceae and Labiatae)

On the basis of study of over 200 species from all over India, it has been found that the members show considerable cytomorphological diversity. Several species with intraspecific races have been identified and the presence of accessory or B-chromosome has been shown in many others. It has been shown



that in the case of Acanthaceae as compared to the Labiatae, polyploidy has not been a potent factor in cytological evolution. The high aneuploid numbers in the two families, at both generic and specific levels, indicate the role of aneuploidy in the evolution of taxa, leading to morphological variations. In the Labiatae<sup>13</sup>, the polyploidy-habit correlations of the Indian members reveal that the perennials (37.03%) and herbaceous (30.06%) members exhibit higher incidence of polyploidy as compared with the annuals (30.73%) and shrubby members (24%).

#### (d) Grasses and Sedges

Because of their economic importance and wide distribution, the grasses and sedges of the Punjab region (nearly 100 species) were subjected to chromosomal analysis; this work has helped (i) to project the existing great variability, and (ii) in the identification, collection and cultivation of different germ plasms. The various genera that turned out to be of cytological interest are *Saccharum*, *Dactyloctenium*, *Lophochloa*, *Echinochloa*, *Cyperus*, *Fimbristylis*, etc.

#### (e) Karyomorphology

Analysis of somatic chromosomes of nearly 300 taxa, most of which are legumes, has led to the following inferences:

(i) Chromosome size is the real determinant of the total chromatin length rather than the chromosome number.

(ii) There is absolutely no correlation between ploidy level and the total chromatin length of the haploid complement.

(iii) There is hardly any correlation between the number of chromosomes with SATs or secondary constrictions and the level of ploidy.

(iv) From among 55 species of weeds of cultivable lands<sup>14</sup>, majority have symmetrical karyotypes. These weeds show mostly meta- or submetacentric chromosomes. Species with secondary constrictions are not many. *Commellina benghalensis* shows the most highly evolved karyotypes.

(v) Among legumes, tree species mostly possess meta- and submetacentric chromosomes (with secondary constrictions on some); among shrubs, there is preponderance of metacentric chromosomes, though acrocentric chromosomes are not rare, whereas in herbs, the majority of the genera are with submetacentric chromosomes. Acrocentric chromosomes may be seen in nearly 50% genera (here secondary constrictions are also not rare). In Caesalpinaceae (Cassiaceae and Mimosaceae), as compared to trees and herbs, the shrubby species seem to have more evolved karyotypes<sup>15,16</sup>.

(vi) Sedges show a great variety of differences in the karyotypes of individuals of different populations<sup>17</sup>. This very well shows that evolution is taking place at 'micro-level'.

#### (f) Cytopalynology

Very little attention has been paid to the correlation of the cytological events with the structural aspects of pollen and spores<sup>18</sup>. With the increasing importance of palynology in reproductive biology, pollen of several hundred taxa of Indian plants from actual cytological vouchers were studied. It has been seen that meiotic

irregularities influence the number, size, shape and ornamentation of the pollen. Woody taxa have small and thin-walled pollen fit for anemophilous pollination so common in them. Interestingly, at species level, there is no correlation between pollen size and ploidy level, but in the case of intraspecific cytotypes, polyploids may have bigger pollen.

## (2) Taxonomy

Studies on the flora of Patiala<sup>19</sup> have resulted in the reporting of a large number of new members. Also, the taxonomy of at least 50 North Indian plant species has been clarified and their nomenclature discussed.

## (3) Distribution, Frequency, Periodicity and Evolution of Weeds of Cultivable Lands

Studies on the weeds of wheat, rice, maize, cotton, sugarcane, oilseeds, etc. have helped in predicting periods when there is maximum crop-weed competition. Such information should enable better management of cultivable lands for optimizing yields.

In Patiala district, nearly 90% of the weeds in crop fields are herbs and out of these about 78% are annuals. Complementary chromosomal data indicated that diploids (61.9%) outnumber the polyploids (38%). In the fields, there is higher percentage of polyploid annuals (36.6%) in comparison to polyploid perennials (only 10%). High incidence of polyploidy (47%) is indicative of the fact that the weed flora of Punjab lying in the subtropical region is in fairly active state of evolution<sup>20</sup>.

A detailed account of the significant contributions of Bir on the cytology of

flowering plants is available in his presidential address to the 'Society for Advancement of Botany' in its annual meeting held at HAU, Hissar<sup>11</sup>.

In addition to the above, the work of Bir on non-vascular plants—the Bryophytes—concerns the systematics of Thallose liverworts of Nainital, Dalhousie and Simla regions in the North Western Himalayas.

## Future Work

In the coming years, studies relating to (i) Biosystematics of the Pteridophytes of the Himalayas, Andamans and Nicobar Islands, (ii) Cytological diversity in the woody taxa (Gamopetalae and Monochlamydeae) of South India and adjacent regions, (iii) Cytological analysis of flora of the plains of North India, (iv) Morphology, palynology and taxonomy of the Asplenioid, Athyrioid and Polypodioid ferns of India and (v) Taxonomic revision of Fern Flora of India, are proposed to be taken up.

## Selected Publications

1. Bir S S, Evolutionary status of the Asplenioid and Athyrioid ferns with particular reference to the Himalayan ferns, *Cytologia*, **37** (1972) 175-96.
2. Bir S S, Cytology of Indian Pteridophytes, *Glimpses P1 Res*, **1** (1973) 28-119.
3. Bir S S & Trikha C K, Cytological evolution of polypodioid ferns with particular reference to the Himalayan forms, *Glimpses P1 Res*, **5** (1978) 98-130.
4. Vasudeva S M & Bir S S, Chromosome numbers and evolutionary status of ferns and fern allies of Pachmarhi hills (Central India), *Asp P1 Sci*, **6** (1982) 119-81.
5. Bir S S, Cytogenetics of Pteridophyta, in *Genetical Research in India*, edited by Jaiswal P L & Wadhvani A M (ICAR, New Delhi) 1983, 46-60.
6. Bir S S, Fraser-Jenkins C R & Lovis J D, *Asplenium punjabense* sp nov and its significance for the status of *Ceterach* and *Ceterachopsis*, *Fern Gaz*, **13** (1985) 53-64.

7. Mehra P N & Bir S S, Pteridophytic flora of Darjeeling and Sikkim Himalayas, *Res Bull (N S) Punjab Univ*, **15** (1964) 69-182.
8. Bir S S, Satija C K, Vasudeva S M & Goyal Parmod, *Pteridophytic flora of Garhwal Himalayas (Mussorie, Dehradun and adjoining hills)* (Jugal Kishore & Co, Dehradun), 1982, i-xii, 1-83.
9. Satija, C K & Bir S S, Polypodiaceous ferns of India, *Aspects of Plant Sciences*, Vol 8 (Today & Tomorrow's Printers & Publishers, New Delhi), 1985, pp. 126.
10. Bir S S, Gill B S, Bedi Y S & Singhal V K, Evolutionary status of the woody taxa of Garhwal Himalayas, in *Improvement of forest biomass*, edited by P K Khosla (Indian Soc Tree Scientists, Solan), 1982, 81-96.
11. Bir S S, Reminiscences of a cytologist, *Acta bot Indica*, **11** (1983) i-xxi.
12. Bir S S & Kumari S, Evolutionary status of Leguminosae of Pachmarhi, Central India, *Nucleus*, **20** (1977) 94-98.
13. Saggoo M I S & Bir S S, Cytological evolution in the Indian Labiatae, *Trends Pl Res* (1985) 367-396.
14. Sidhu M & Bir S S, Karyological studies on weeds of cultivable lands in Punjab, India, *Trop Pl Sci Res*, **1**(1) (1983) 1-13.
15. Bir S S & Kumari S, Karyotypic studies in *Cassia* Linn. from India, *Proc Indian natn Sci Acad*, **B48** (1982) 397-404.
16. Kumari S & Bir S S, Karyomorphological evolution in Mimosaceae, *J Cytol Genet*, **20** (1985) 16-35.
17. Bir S S, Sindhu M & Kamra S, Karyotypic studies on certain members of Cyperaceae from Punjab, North West India, *Cytologia*, **51** (1986) 511-22.
18. Saggoo M I S & Bir S S, Cytopalynological studies on Indian members of Acanthaceae and Labiatae, *J Palynol*, **19** (1983) 243-77.
19. Sharma M & Bir S S, *Flora of Patiala* (Punjabi University, Patiala) 1978, pp i-xii, 240.
20. Bir S S & Sidhu M, Evolutionary status of weed flora of cultivable lands in Punjab, India, in *Current approaches in cytogenetics*, edited by R P Sinha and U Sinha (Spectrum Publ House, New Delhi) 1983, 135-44.



## M N Bose

Having reached the evening of my life, when I assess my scientific career, I feel that I was a sort of driftwood. After graduation, I joined a medical college. That subject was not to my liking, so I left (I have never forgiven myself for that wrong decision). Then I joined for MSc in botany. Prof. Birbal Sahni's lectures had a deep effect on me. That led me to join research in palaeobotany. After final deposition, viz., after finally opting for research in palaeobotany, I have been publishing on the average 4-5 papers every year.

Prof. Sahni, soon after accepting me as one of his research students and appointing me as his personal research assistant, left for USA for about a year. After his return he got busy with the foundationstone laying ceremony of the Palaeobotany Institute and unfortunately, soon after on 10 April 1949, he passed away. So I got very little guidance from him and was left without a research guide. I struggled hard and compiled my PhD thesis after three years, taking help off and on from some palaeobotanists senior to me. The work was mainly based on some fossil plants from the Jurassic of the Rajmahal Hills and some angiospermic remains from the Tertiary of Rajasthan.

Out of my PhD thesis I published quite a few papers. The paper on a species of *Bucklandia*, belonging to Bennettitales, was very well accepted by palaeobotanists

all over the world. It gave convincing evidence that the genus *Homoxylon*, described by Prof. Sahni as an angiospermic wood from the Jurassic of the Rajmahal Hills, was a bennettitid wood. It also gave another example of the extraordinary parallel evolution of wood structure, which occurs here and there throughout the vascular plants. The other papers included work on petrified bennettitid leaves and some conifer remains from the Rajmahal Hills. From Rajasthan, angiospermic remains belonging to the family Guttiferae were described. Since then remains of Guttiferae were found by various Tertiary palaeobotanists all over India. Surprisingly enough, most of the work I did in connection with my PhD thesis even now is often quoted. I must confess it is all because of chance findings.

Soon after my PhD, I went to England and Europe and there I worked with Prof. T M Harris, FRS (Reading University), Prof. Hamshaw Thomas, FRS (Cambridge University), Prof. O H Selling (Riksmuseum, Stockholm) and Prof. Ove Arbo Hoeg (University of Oslo). I learnt real palaeobotany under Harris (a remarkable person, a real scientist who lived and died for science. He never cared for administration or aspired to be a High Priest of science or wanted to be chairman of various committees and be like a

---

Formerly, Director, Birbal Sahni Institute of Palaeobotany, Lucknow; Residence : B 965, Sector A, Mahanagar, Lucknow-226006.

porcelain Budha for decoration on mantlepieces. He never imagined he could produce PhD students on a production line, having 30-40 students at a time working under him. He never had more than one or two PhD students at a time). At Reading, I worked on some specimens from the Jurassic of Yorkshire. One paper was on *Pachypteris* and two on conifers (*Farndalea* and *Taxulus*). Among the so-called Mesozoic pteridosperms the species of *Pachypteris papilosa* is now very well known all over the world. From Harris, besides palaeobotany, I learnt many other things. One of the things he told me was to never compromise on facts. I have been following it strictly and have paid heavily for that.

At Riksmuseum, Stockholm, I worked on plants collected from the Upper Cretaceous of New Zealand and Eocene of Queensland, Australia. From New Zealand, *Araucaria haastii* was described, which had a surprising resemblance with the extant species *A. klinkii*. Australian plants were remains of shoots and cones of *Athrotaxites*.

Prof. Hoeg had a collection of Palaeozoic fossil plants from Belgian Congo (now known as Zaïre) and Zambesi (Mozambique). The flora was worked out jointly with Prof. Hoeg and it resulted in a big monograph on fossil plants ever published from Zaïre. While working on these plants, I did bulk maceration of some shale samples from the Palaeozoic of Luena and other localities. During the study of the macerates, under the microscope, I came across some megaspores which had a peculiar inner body (mesosporium). We published a paper on these megaspores and this opened a new line of research for the

megaspore workers. I personally did not pursue this work, but I learnt that palaeobotanists and palynologists should do maceration and scanning of their samples themselves. Because of the work on the fossil flora of Congo, I was requested by the Director, Musée Royal de l'Afrique Centrale, Tervuren (Belgium) to visit Zaïre and make fresh collections. After my return from Zaïre I worked on those collections ranging in age from Palaeozoic to Upper Cretaceous. In all more than 20 papers were published and those included several monographic works. Based on our palynological work, the Belgian geologists had to change the classification of the Lukuga Group and some Mesozoic formations in Zaïre.

Another paper which I published, while I was in Oslo, was on some leaves of *Sciadopitys* from the Arctic of Canada. That work threw some light on the 'double needle theory', though the matter is still controversial.

Returning to India, I confined myself mainly to the Mesozoic fossils. My work ranged in age from Lower Triassic to Lower Cretaceous. In the Triassic, which was then very little known in India, many new localities were discovered. Three distinct floral assemblages belonging to Lower, Middle and Upper Triassic were distinguished. Formerly, the genus *Dicroidium* was supposed to be a Triassic marker in India. Our work showed that the genus *Lepidopteris* was actually the Permo-Triassic marker in India. From Nidhpur two interesting cones were described. One of them, *Nidia*, matched very much the female cones of the extant genus *Zamia*.

From the Jurassic-Lower Cretaceous many fossil plants new to Kachchh,



Saurashtra, Gardweshar (Gujarat), South Rewa and Satpura basins, the Rajmahal Hills and East Coast Gondwanas were described. Wherever possible, comments were made, on the basis of plant assemblages on the age of the various formations. Due to the presence of *Weichselia* and *Onychiopsis* undoubted Lower Cretaceous beds were recognized at Bansa, Himmatnagar and Than. A monograph on the Mesozoic fossil flora of Kachchh has been published which has set an example for other monographs which need to be published, viz., Fossil flora of the Rajmahal Hills, Lower Cretaceous floras of India, etc. Besides, smaller monographs have been published on the pteridophytes from the Rajmahal Hills, Cycadophytic leaves from Jurassic-Lower Cretaceous rocks of India, bennettitalean leaves like *Otozamites*, *Ptilophyllum* and *Dictyozamites* and bennettitalean male and female fructifications like *Weltrichia* and *Williamsonia*. My recent papers include the work on a bisexual bennettitalean flower, *Amarjolia dactylota*. It is the only bisexual bennettitalean flower so far known from India. It will be useful for teaching purposes. The other paper which will also be useful for teaching purposes is on 'The *Pentoxylon* plant', jointly published with Dr P K Pal and Prof. T M Harris. It has changed some of the old concepts.

Besides working on the Mesozoic plants from Peninsular India I have also worked on fossil plants collected from the Himalaya. A paper published on the Upper Palaeozoic formations from Kashmir Himalaya has shown that during the Lower Carbonaceous, the flora was similar to those described from the northern hemisphere and other parts of the World. Also, the Permian flora had elements of

Chinese flora. Four papers on the Mesozoic plant remains from Bhutan, Nepal and Ladakh have also been published. It was found that whereas the assemblages from Bhutan and Nepal were somewhat similar to the ones known from the Rajmahal Hills and Jabalpur Formation, the assemblage from Ladakh was entirely different. It resembled the ones known from the northern hemisphere.

In the end I am happy to record that I have mostly worked on fossils which I had collected myself. My field trips extended from the equatorial forests of Zaire to Spitsbergen about 10° south of North Pole and from plains of Peninsular India to about 18,000 ft in the Himalaya. I was quite often lucky enough to get new things. So my success was more accidental than due to the result of careful planning. After my retirement I miss the field trips (I had been to Kachchh for collection of fossil plants even a month before my retirement). I wish I could take young students to the fields—teach them palaeobotany and how to collect fossils right at the locality itself. Alas I have no funds to do so.

I have published nearly 150 papers, including quite a few monographs, but frankly I have got real satisfaction or pleasure out of only 8-10 papers.

### Selected Publications

1. Bose M N, *Bucklandia sahnii* sp nov from the Jurassic of the Rajmahal Hills, Bihar, *Palaeobotanist*, **2** (1953) 41-50.
2. Bose M N, *Sciadopitytes variabilis* n. sp. from the Arctic of Canada, *Norsk Geol tids*, **35** (1955) 53-68.
3. Hoeg O A, Bose M N & Manum Svein, On the double walls in fossil megaspores with description of *Duosporites congoensis* n. gen., n. sp. *Nytt Mag F Botanikk*, **4** (1955) 101-6.



4. Hoeg O A & Bose M N, The *Glossopteris* flora of the Belgian Congo with a note on some fossil plants from the Zambesi Basin (Mozambique), *Ann Mus Roy Congo, Belge*, **8** (1960) 1-107.
5. Bose M N, Fossil<sup>1</sup> plant remains from the Rajmahal and Jabalpur Series in the Upper Gondwana of India, *Symp Flr Strat Gond*, (1966) 143-54.
6. Bose M N & Kar R K, Palaeozoic *Sporae Dispersae* from Congo. I—Kindu-Kalima and Walikale regions, *Musée R Afr Cent*, **8** (53) (1966) 1-238.
7. Bose M N, A new species of *Williamsonia* from the Rajmahal Hills, India, *J Linn Soc (Bot)*, **61** (384) (1968) 121-27.
8. Sitholey R V & Bose M N, *Weltrichia santalensis* (Sitholey & Bose) and other bennettitalean male fructifications from India, *Palaeontographica*, **131** (5-6) (1971) 151-59.
9. Bose M N & Kasat M L, The genus *Ptilophyllum* in India, *Palaeobotanist*, **19** (2) (1972) 115-45.
10. Bose M N, Triassic floras, *Aspects and Appraisal of Indian Palaeobotany*, (1974) 285-93.
11. Bose M N & Kar R K, Biostratigraphy of the Lukuga Group in Zaïre, *Annls Mus R Afr Cent Ser 8°*, *Sci geol*, **82** (1978) 97-113.
12. Gopal Singh, Maithy P K & Bose M N, Upper Palaeozoic floras of Kashmir Himalaya, *Palaeobotanist*, **30** (2) (1982) 185-232.
13. Bose M N, Banerji Jayasri & Pal P K, *Amarjolia dactylota* comb nov, a bennettitalean bisexual flower from the Rajmahal Hills, India, *Palaeobotanist*, **32** (3) (1984) 1-189.
14. Bose M N & Banerji Jayasri, The fossil floras of Kachchh. I. Mesozoic megafossils, *Palaeobotanist*, **33** (1984) 1-189.
15. Bose M N, Pal P K & Harris T M, The *Pentoxylon* plant, *Phil Trans R Soc*, **B310** (1985) 77-108.

## V L Chopra

A brief account of the major contributions of Chopra and his colleagues is given below.

(1) *Indirect Radiation Effects* : An irradiated medium has significant biological effects at the cellular level as measured through mutagenesis in bacteria and inhibition of cell division, chromosome breakage and other cytological abnormalities in plants. At organismic level, the genetic effects are marginal.

(2) *Cytological Effect of Chemical Mutagens* : Mutagens like ethyl methane-sulphonate show a strong preferred reactivity in the centric region of wheat and barley chromosomes and can be usefully employed for creating variability in a region protected from crossing over by centromeric interference.

(3) Induced mutagenesis technique has been employed to probe the phylogenetic relationship in the genus *Triticum*. Isolation of sphaerococcoid mutants allelic with 'S' locus of *T. sphaerococcum* and branched ear mutants phenotypically sharing characteristics with *T. turgidum* has suggested that macromutations have played a significant role in speciation in the Triticinae.

(4) Treatment conditions and protocols have been developed which substantially alter the yield and spectrum of mutations in crop plants induced by radiations and chemicals individually or in combination.

(5) A complete series of 21 monosomic lines has been developed in the Indian bread wheat variety Pb. C-591.

(6) Through mutagenesis, rust resistant mutants have been induced in the wheat cultivars Kharchia Local and Lal Bahadur. The induced mutants in Kharchia Local show complete freedom from infection against all brown rust races available in India and against laboratory constructed virulences in Australia, Canada and USA. Based on this unusual observation, the hypothesis has been advanced, and put to experimental test, that the induced mutation has either created a new locus for host resistance against which a matching virulence of pathogen is not available or alternatively, the induced mutation has affected a control locus. The induced mutations for resistance in the variety Lal Bahadur have been put to extensive yield test under farmers' field conditions in the area of their adaptation (Rajasthan) and have shown consistently good performance, meriting their release for large scale cultivation.

Mutants for amber grain character have also been induced and evaluated in the

variety Tonari 71. Induced mutations showing freedom from grain mottling in the variety Arjun (HD 2009) have been isolated. In yield test, the mutants have shown performance at par with that of the respective parental variety.

(7) Information on the effectiveness of known *Sr* genes for resistance to black rust and *Lr* genes for resistance to leaf rust against Indian virulences, not hitherto available, has been obtained and documented.

(8) An analysis for the content of specific genes for host resistance against rusts have been made in some wheat varieties and genetic stocks and their origin has been traced to prospective donors.

(9) Genetic analysis of slow leaf-rusting resistance in wheat has led to the following conclusions:

(i) The fast-rusting genotypes have a shorter latent period, produce larger uredia which have more spores per uredium per day than the slow-rusters at the adult plant stage. Correlation analyses indicate that spores produced per uredium per day and the rate of increase in uredium area are important components and together they account for 74% of variation for slow-rusting resistance in the field as measured in terms of area under disease progress curve. The analysis also indicates that all components and slow-rusting resistance are predominantly under the control of additive gene action.

(ii) In order to overcome the difficulty of extending glasshouse data (based on a single cycle of infection) to field performance of slow-rusting resistance (a polycyclic situation), the validity of Shaner and Hess equation has been determined by

comparing the hypothetical with the observed disease progress curves of 15 genotypes of wheat. It has been observed that the observed values of the area under disease progress curve (AUDPC) for a majority of the genotypes are fairly close to their respective hypothetical AUDPC values. It should, therefore, be possible, using Shaner and Hess equations, to predict slow-rusting resistance of cultivars fairly exactly in glasshouse studies.

(iii) The relative importance of components (latent period, uredium area, infectious period, infectibility, sporulation and weather) affecting the progress of leaf rust was assessed from equivalent changes in the individual components. Only four components, viz. latent period, infectibility, sporulation and weather, were found to be important. It was also found that these four components were equally important and that they collectively determined the rate of progress of leaf rust in an additive manner.

Our work has helped us in developing concepts and generating information, on the basis of which it is now possible to give a scientific direction to breeding for more durable kind of resistance.

(10) Wheat varieties DL 20-9, for Northern Zone, and HW 657 for Peninsular Zone, developed in our project, have been identified for release.

(11) In our research project "Breeding Improved *B. campestris* var Sarson", a number of improved populations and strains have been developed which have shown consistently good performance in the All India Coordinated Trials and on farmers' fields in the trials conducted by the Vanaspati Manufacturers' Association.



(12) Mutants of *Drosophila melanogaster* affecting developmental (Wingless,  $wg^1$ ) and behaviour (Hyperkinetic) characteristics have been induced and characterized. They have shown several novel features.

(13) The predominantly acrocentric somatic chromosome complement of the mouse associates into ring bivalents at meiosis. This is an unusual feature. From our study of the two types of cell division in the mouse, we have advanced an explanation accommodating the two contradictory observations.

(14) In *Escherichia coli*, single gene control of complete and partial mutational stability has been demonstrated. The 'ad' mutation, for example, stabilizes *Trp* 6 locus completely against spontaneous and induced reversions. The *thr* mutation, on the other hand, inflicts only partial blocks, so that while spontaneous *trp* reversions are not obtained, with mutagenic treatments a low frequency is obtained.

(15) A large number of mutator mutations in *E. coli* with properties and locations different from those of the known mutators have been induced, characterized and located.

(16) The differential mutability of two markers (*met trp*<sup>-</sup>) by different mutagens has been studied in detail and has been established to be a case of true mutagen specificity.

(17) In *Azotobacter chroococcum*, notoriously unyielding of stable biochemical markers, a number of stable mutations for amino acid and DNA base requirements, drug resistance and nitrogen fixation have been induced and characterized. A stage has now been reached when formal genetics of this

agriculturally important genus will soon be possible.

### Selected Publications

1. Swaminathan M S, Chopra V L & Bhaskaran S, Cytological aberrations observed in barley embryos cultured in irradiated potato mash, *Radiat Res*, **16** (1962) 182-88.
2. Swaminathan M S, Chopra V L & Bhaskaran S, Chromosome aberrations and the frequency and spectrum of mutations induced by ethyl methane sulphonate in barley and wheat, *Indian J Genet*, **22** (1962) 192-207.
3. Chopra V L, Natarajan A T & Swaminathan M S, Cytological effects observed in plant material grown on irradiated fruit juices, *Radiat Bot*, **3** (1962) 1-6.
4. Chopra V L, Tests on *Drosophila* for the production of mutations by irradiated medium and irradiated DNA, *Nature, Lond*, **208** (1965) 699-700.
5. Chopra V L & Pai R A, Chromosomes of the striped Indian squirrel (*Funambulus penenti*), *Nature, Lond*, **207** (1965) 1110.
6. Chopra V L, Gene controlled mutational stability of tryptophanless mutant of *E. coli* WP-2, *Mutation Res*, **4** (1967) 382-84.
7. Chopra V L, Lethal and mutagenic effects of irradiated medium in *E. coli*, *Mutation Res*, **8** (1969) 23-33.
8. Chopra V L, DNA feeding and "Directed mutagenesis" in *Drosophila melanogaster*, *Genet Res, Camb*, **18** (1970) 345-46.
9. Sharma R P & Chopra V L, Innate metabolic differences and mutagen sensitivity of *Drosophila melanogaster*. I. Radio-sensitivity of hyperkinetic mutant, *Mutation Res*, **30** (1) (1975) 77-82.
10. Sharma R P & Chopra V L, On the time and mode of action of wingless ( $wg^1$ ) gene during wing and haltere differentiation in *Drosophila melanogaster*, *Develop Biol*, **48** (1976) 461-65.
11. Sawhney R N, Nayar S K, Singh S D & Chopra V L, Virulence pattern of the Indian leaf rust races on lines and varieties of wheat with known *Lr* genes, *SABRAO J*, **9** (1) (1977) 13-20.
12. Sawhney R N, Chopra V L & Swaminathan M S, Analysis of genes for resistance against Indian stem rust races in two bread wheat varieties, *Euphytica*, **28** (1977) 651-60.

13. Kulkarni R N & Chopra V L, Slow rusting resistance: Its components, nature and inheritance, *Z Pflanz Pflanz*, **87** (9) (1980) 562-73.
14. Kulkarni R N, Chopra V L & Daljit Singh, Observed and hypothetical leaf rust progress curves of some genotypes of wheat, *Theor appl Genet*, **60** (1981) 85-88.
15. Kulkarni R N, Chopra V L & Daljit Singh, Relative importance of components affecting leaf rust progress curves in wheat, *Theoret appl Genet*, **62** (1982) 205-7.
16. Aqbal Singh & Chopra V L, Interaction of mutation with mutationally stable genetic system in *E. coli*, *Mutat Res*, **143** (1985) 191-93.
17. Kashyap L R & Chopra V L, Preponderance of tryptophan auxotrophy in *Azotobacter* by nitrosomethyl urea, *Indian J exp Biol*, **23** (1985) 658-60.

## S N Das Gupta

Plant pathology was in its infancy when Das Gupta joined the Imperial College of Science and Technology, London University, in 1927, for research in this discipline. The immediate problem was saltation (mutation) in several disease-producing fungi of apple fruits, *Cytosporina*, *Phomopsis* and *Diaporthe*.

### Saltation in Fungi

Das Gupta made wide ranging contributions in this field involving commonly observed sectorial saltation, in which the saltants varied in their points of origin, in their shape, size and other morphological characters, as well as in their physiological behaviour. Besides sectorial saltation, Das Gupta came across several little known phenomena of outstanding interest.

(a) Latent (marked) saltation that expresses its identity only when separately subcultured and continues to breed true in successive generations.

(b) Eversaltating strain in which the parent form repeats the phenomenon in every cultural generation as orthogenetic saltation.

(c) Cyclic saltation in which the saltant in some later generation reverts to the parental form.

(d) Conversion of parental strain in which a strain of *Diaporthe*, while retaining its parental character in the hyphae of the advancing region, undergoes conversion into a saltant strain in the older region of the mycelium.

(e) Faster growth rate of the saltant hyphae than that of the parental hyphae, which may be correlated with greater pathogenic activity.

(f) Inhibition of the parental hyphae caused by the saltant hyphae.

(g) A phenomenon of profound interest is the saltation into complementary strains in *Cytosporina*. Such saltants, separately infertile when grown adjacently in nutritive medium, may form pycnidia along their line of junction; or form scattered pycnidia when the hyphae of the two grow intermingled with each other. This is a form of a sexual heterothallism.

(h) Yet another phenomenon of profound interest, particularly from the point of view of plant diseases, is the production of saltants of different degrees of pathogenicity. Some of these are more virulent than the parent strain, demonstrating that the origin of more virulent strains from weaker parent may not be uncommon in nature, thus posing problems for plant disease control.

---

Formerly, Vice-Chancellor, University of Kalyani, West Bengal; Residence : 1-B Judges Court Road, Calcutta-700027.



On his return to India, and with his appointment in the Lucknow University, as Reader in Botany in 1934, it was the endeavour of Das Gupta to develop the department into a prominent centre of research in mycology and plant pathology, in which he received the fullest cooperation and encouragement from Prof. Birbal Sahni, one who made it an unparalleled centre for researches in palaeobotany in India.

### **Fungal Diseases of Economic Plants**

Das Gupta initiated his research students into researches on problems of agricultural interest. These involved diseases of bajra, sawan, guava, mustard, malformation of mango blossoms, soft rot and other mango diseases, die-back of mango tree, latent infection in mango and *Bel* fruits, soil-borne diseases, such as wilt of guava, etc., storage diseases and other such diverse problems.

### **Utilization of Usar Soil**

Researches were also carried out on the Usar soil of UP. Usar soil, that owes its origin to climatic factors throughout the ages, hardly lends itself to permanent reclamation. The possibility of its utilization by the regeneration of some types of grasses in irrigated Usar soil was demonstrated convincingly.

### **Air Pollution and Mango Necrosis**

Das Gupta investigated the problem of air pollution causing necrosis of mango fruits in orchards adjacent to the operating brick-kilns through coal fumes emanating from them. The problem was tackled from various angles.

The non-pathogenic nature of the necrosis was proved conclusively, as no causal, parasitic or infective agent capable

of reproducing the disease could be isolated. Further, this physiological disease could not be reproduced experimentally by fumigation with coal fumes and its constituents—sulphur dioxide, ethylene, etc. Injection of crude extract of the necrotic tissues also did not produce the disease. The causal factor was demonstrated to be a fraction of ether soluble constituents of brick-kiln fumes, also soluble in chloroform, that crystallizes in hexagonal form (mp  $11.05^{\circ} \pm 0.5^{\circ}\text{C}$ ). This, when injected, produced necrosis in 50% of the experimental fruits under controlled conditions. A similar fraction was found in the crude mango extract.

Spraying of boron at a certain concentration at the blossoming stage and early stage of development of the fruits prevented and arrested the development of the disease, but it did not cure the disease once it had been produced. Successful prevention of the disease indicates that the disease producing fraction of the fumes when absorbed by the fruits induces boron deficiency. It also causes histopathological changes, forming deposits in the vessels, choking, impeding or altogether stopping the translocation of metabolic products to the distal end, thus causing necrosis in the tip region that gradually progresses towards the shoulder region.

These problems need reinvestigation at a much wider scale; this could be done by Das Gupta with the woefully inadequate men, materials and financial grant from the ICAR and the state agricultural department in those pre-independence and early post-independence days.

### **UNESCO Interlude**

There was an interlude in these research activities during the period 1946-1949,

when Das Gupta left for Paris to serve UNESCO as a Counsellor in Agricultural Sciences. He was called back, when the Professorship fell vacant with the premature death of Prof. Birbal Sahni.

### The Second Phase (1950-1958)

Plant pathology in the department was revitalized by the introduction of two then modern disciplines, virology and deficiency diseases (trace element studies) with the help of two lecturers already trained abroad for the purpose. Das Gupta himself pursued the air pollution work, yet more conscious of the gravity of this national problem on his exposure to the UNESCO ideal, and further initiated researches in two branches of mycology—Medical mycology and aquatic phycomycetes. Both these had to be terminated as the specialists dispersed and Das Gupta himself left for Calcutta as a member of the Public Service Commission, West Bengal, as a prelude to the position of foundation Vice-Chancellor of Kalyani University, West Bengal (1960-1968).

### Medical Mycology

Important contributions were made on the mycotic diseases of man and dermatophytic fungi were studied from the mycological point of view. A comprehensive review of medical mycology in India was published simultaneously.

### Aquatic Phycomycetes

More than 70 species have been discovered, most of them new to science, distributed over five orders, 10 families and 16 genera. Of these, very few have been published and the rest are under revision. The majority, however, are ready for immediate publication. The genera

represented are: *Olpidium*, *Rozella*, *Phlyctidium*, *Rhizophyidium*, *Blyttimyces*, *Entophlyctis* and *Rhizidia* (inoperculate chytridiales); *Chytridium macrochytrium* and a proposed new genus *Sparrowmyces* (operculate Chytridiales); *Catenaria*, *Allomyces* and *Blastoclada* (Blastocladiales); *Gonapodya* (Monoblepharidales); *Olpidiopsis*, *Myzocyttium* and *Lagenidium* (Lagenidiales); and *Ancylistes* (Entemophthorales).

Such huge and varied collection from a single laboratory, within a short span of five years, is indeed phenomenal, and is surpassed only by Sparrow Karling and perhaps Scherffel, their collection extending over many years.

### Selected Publications

1. Das Gupta S N, Saltation in fungi, *Lucknow University Studies*, No V, 1936.
2. Das Gupta S N, History of mycology and plant pathology in India, Burma and Ceylon, *J Indian bot Soc*, (1946).
3. Das Gupta S N, Shome S K & Majumdar S S, Medical mycology in India, *Mycopath Mycol appl*, **13** (1960) 339-76.
4. Das Gupta S N, Air pollution in relation to plant diseases, *Presidential Address, Botany Section, 44th Indian Science Congress*, 1957.
5. Das Gupta S N, Discourses on aquatic phycomycetes of India, *Indian Phytopath*, **35** (1982) 193-216.
6. Horne A S & Das Gupta S N, On the occurrence of an eversaltating strain in *Diaporthe pernicioso*, *Ann Bot*, **43** (1929) 417-35.
7. Das Gupta S N, On the occurrence of saltation in *Cytosporina* and *Diaporthe*, *Ann Bot*, **44** (1930) 349-84.
8. Das Gupta S N, On the pathogenicity of *Cytosporina* and its saltants, *Ann Bot*, **47** (1933) 197-226.
9. Das Gupta S N, On the pathogenicity of certain strains of *Phomopsis* and *Diaporthe*, *Ann Bot*, **47** (1933).
10. Das Gupta S N, Formation of pycnidia in *Cytosporina ludibunda* by the intermingling of two infertile strains, *Ann Bot*, **47** (1933).



11. Das Gupta S N, On the conversion of one strain of Diaporthe by another, *Phil Trans R Soc, Ser B*, **497** (1934).
12. Das Gupta S N & Verma G S, Preliminary observations on the necrosis of the mango fruit with special reference to the external symptoms of the disease, *Proc Indian Acad Sci*, **9** (1939) 23-28.
13. Das Gupta S N & Sinha S, Investigations on the effect of sulphur dioxide gas on the mango fruit, *Proc Indian Acad Sci*, **13** (1941) 71-83.
14. Das Gupta S N, Investigations into the pathological histology of fruits affected with black-tip disease with a note on the anatomy of the fruit, *Proc natn Acad Sci India*, **14** (1944) 102-3.
15. Das Gupta S N & Asthana S N, Histopathology of the necrotic mango fruit, *Curr Sci*, **13** (1944) 77.
16. Das Gupta S N, Asthana S N & Bhatt R S, Occurrence of deposits in necrotic mangoes, *Indian J agric Sci*, **25** (1955) 237-52.
17. Das Gupta S N, Verma G S, Agarwal S C, Rai J N & Iyer S N, *Curr Sci*, **19** (1950) 133.
18. Das Gupta S N, Iyer S N & Verma G S, Isolation of a brick-kiln fume constituent causing mango necrosis, *Indian J agric Sci*, **26** (1956) 259-66.
19. Das Gupta S N & Sen C, On the prevention of mango necrosis, *Curr Sci*, **27** (1958) 446-47.
20. Das Gupta S N, Further studies in the effect of boron on mango necrosis, *Proc natn Inst Sci India*, **26** (1960) 80-87.
21. Das Gupta S N, On the effect of boron on mango necrosis, *Phytopathology*, **50** (1960) 432-33.
22. Das Gupta S N, Discourses on aquatic phycimycetes of India, *Indian Phytopath*, **35** (1982) 193-216.
23. Das Gupta S N & Rachel John, Studies in the Indian aquatic fungi. I, Some water moulds of Lucknow, *Proc Indian Acad Sci*, **38** (1953) 165-70.



## T V Desikachary

Desikachary took his MSc degree in 1944 and PhD degree of the University of Madras in 1951, based on his researches on morphology and taxonomy of blue green algae under the guidance of late Prof. M O P Iyengar. He spent a year (July 1953-May 1954) at the University of California, Berkeley, doing research work on the red algae with Prof. G F Papenfuss. He also undertook a tour of research and field collection of marine algae in Indonesia, New Zealand and Australia under the sponsorship of the Nuffield Foundation, during April-July 1955. In 1963, he obtained the DSc degree from the University of Madras. He was appointed Professor at the University of Madras in 1964.

He attended many international seminars and conferences, such as the International Botanical Congress at Paris in 1954 and at Edinburgh in 1964. In May 1969, he visited the United Kingdom on a Research Programme sponsored by the British Council, the UGC and the University of Madras. He also took part in the International Symposium on Blue Green Algae at Kastanienbaum, Switzerland in July 1969. Between September 1973 and February 1974, he was once again sponsored by the British Council, UGC and the University of Madras for a research programme at the British Museum, London, the Cytogamic

Museum, Paris and other centres of phycological research. He worked at the British Museum again in 1977 as an INSA/Royal Society Exchange Fellow. He visited algal laboratories in USA for short periods during 1976, 1979 and 1982. He visited some universities in Australia again in 1984. He is now a Professor Emeritus at the University of Madras.

He was elected a Fellow of the Indian Academy of Sciences (1956), the Indian National Science Academy (1966) and the Phycological Society, India.

He was the Chief Editor of *Phykos* up to 1973. He was also on the editorial boards of *Phycologia*, *Indian Journal of Marine Sciences*, and *Hydrobiologia*. He was a member of the Editorial Board of ICAR for the publication of monographs of Indian algae. He was elected a member of the International Nomenclatural Committee on Algae in 1959 and continued to function in that capacity till 1980.

He was awarded the Sir C V Raman Medal by the University of Madras in 1959. The Indian Botanical Society awarded him the Prof. V Puri Medal in 1980 in appreciation of his service to Indian botany, especially morphology and taxonomy of algae. A Commemorative Volume of *Phykos* was published in his honour in 1984.

Desikachary was associated with many universities and was on the Executive Council of the Central Salt and Marine Chemicals Research Institute, Bhavnagar from 1965 to 1973. He organized two symposia on Algal Taxonomy in 1969 and 1974 at the Madras University, thus bringing about an interaction between leading phycologists from other lands and younger Indian phycologists. He has published a large number of research papers on diverse groups of algae. The books published or edited by him include: (1) A monograph on the 'Cyanophyta' (ICAR, 1959), (2) 'Taxonomy and Biology of Blue Green Algae' (University of Madras, 1972), (3) 'Marine Plants' (NCERT, 1975), (4) 'Volvocales' (ICAR, 1981, jointly with Prof. M O P Iyengar) and (5) 'Taxonomy of Algae' (University of Madras).

The late Prof. M O P Iyengar left behind a large volume of unpublished material, expressing at the time of his passing away a desire that these be published by Desikachary. Accordingly, Desikachary started publishing these as a series entitled "Contributions to our knowledge of South Indian algae" from 1967, humbly confessing while doing so, "It has been an embarrassing duty for me to edit these for the press. Aware of my limitations, I have endeavoured to present his observations to the best of my abilities". He is now engaged in the preparation for publication of Prof. Iyengar's monograph on Siphonales, and has presently a project for studying Indian marine algae under the auspices of DST, Government of India. These studies include study of Indian Ocean diatoms (fossil and living), red algae and brown algae.

Desikachary has been carefully nurturing and building up the phycological tradition left behind by Prof. Iyengar. He established a culture collection of algae at the University of Madras. He and his students made fundamental contributions towards a better understanding of the taxonomy of the algae. He was the first to suggest to his graduate students to use numerical methods in the study of taxonomy of blue green algae and culture techniques for elucidating the widespread phenomenon of polymorphism in the same group. Both these lines of work have had great bearing on many of the vexing problems in the taxonomy of the Cyanophyta. He has also made significant contributions towards elucidation of phylogeny and interrelationships in the Charophytes. Critical studies have been made by him on the morphology, life history and interrelationships of red algae, with particular reference to the Nemalionales and the Coralline red algae. His major taxonomic contributions include the establishment of several new orders and families in diverse groups of algae, as also genera commemorating Prof. Iyengar and other illustrious figures in phycology: *Iyengariella* (Cyanophyceae); *Iyengario-monas*; *Papenfussiomonas*, *Schillerio-monas* and *Mantoniella* (Chlorophyceae, Prasinophyceae) and *Rossiella* (Bacillariophyceae). He published many new genera and species left unpublished by the late Prof. Iyengar. His contributions in the field of theoretical phycology are no less outstanding. He has amplified Prof. Iyengar's hypothesis regarding the origin and evolution of the filamentous habit and also postulated that the so-called parenchymatous thalli in many algae are modifications of the palmelloid habit. The observations that in the 'truly'



parenchymatous tissues, plasmodesmata between adjacent (and genetically related) cells get dissociated prior to cell division, leading to vegetative and sexual reproduction and prior to meiosis, has led him (along with Prof. Swamy) to postulate that isolation and insulation are key processes in the vital phenomena of sexual reproduction and reduction division as well as control or lack of control of differentiation in integrated living systems as also the survival of genetically altered cells, such as zygotes, meiocytes, *in situ* mutants, etc. More recently, he contributed a reinterpretation of the role of auxiliary cells in the red algae.

### Selected Publications

1. Desikachary T V, *Cyanophyta* (ICAR, New Delhi) 1959.
2. Desikachary T V & Sundaralingam V S, The affinities and interrelationships of the Characeae, *Phycologia*, **2** (1962) 9-16.
3. Desikachary T V & Swamy B G L, Isolation and insulation of special cells, *Curr Sci*, **45** (13) (1976) 485-86.
4. Desikachary T V, On the nature of auxiliary cells in red algae, *Seaweed Res Utiln*, **5** (2) (1982) 49-51.
5. Iyengar M O P & Desikachary T V, *Volvocales* (ICAR, New Delhi) 1981.



## H C Gangulee

Gangulee had his initiation into research in embryological cytology under Dr I Banerjee of Calcutta University (1934-35). He got training in wheat genetics research at the Punjab Government Agricultural College and Research Institute, Lyallpur (1936-37). He subsequently worked under the guidance of Prof. P. Maheshwari at Dacca University (1940-42). He worked on rice genetics and breeding in USA (1947-49) as an overseas scholar and then at the Presidency College, Calcutta (1950-56). One of his major contributions in this field was the finding that *Aman* rice is a distinct photoperiodic group of Indian rice. He introduced the technique of hot water emasculation of rice for the first time in India.

From 1956 onwards, Gangulee's main interest was in taxonomy and cytology of Indian mosses. He published his monograph on the mosses of Eastern India and the Adjacent Regions from 1969 to 1980.

### Selected Publications

1. Banerji I & Gangulee H C, Spermatogenesis in *E. erassines* Solms, *J Indian bot Soc*, **16** (1937), 289-95.
2. Gangulee H C, On the development of the embryosac and the pollen grain in *C ensiformis* D C, *J Dep Sci Calc Univ*, **1** (1938) 73-97.
3. Maheshwari P & Gangulee H C, Development of the embryosac in *H. umbellata* Kunth, *J Indian bot Soc*, **27** (1948).
4. Gangulee H C, Rice cultivation in USA, *J Sci Club*, **7**, 130-35.
5. Gangulee H C, Types of flowering behaviour in rice, *O sativa* and the distinctiveness of the *Aman* type, *Curr Sci*, **23** (1954) 80-81.
6. Gangulee H C, Rice anthocyanin genetics. I, *Portugal Acta Biol*, **A4** (1954) 99-134.
7. Gangulee H C, Anthocyanin genetics in plants, with special reference to rice, *Bull bot Soc Beng*, **8** (1956) 156-67.
8. Gangulee H C, Studies on the date of ear emergence in rice. I, Relation between sowing time and date of ear emergence, *Bot Gaz*, **117** (1955) 1-10.
9. Gangulee H C, Rice anthocyanin genetics. II, *Bull bot Soc Beng*, **9** (1955) 121-26.
10. Gangulee HC, Hot water encapsulation of rice, *Sci & Cult*, **21** (1956) 739-41.
11. Gangulee H C, Mosses of Eastern India. I, II, III, *Bull bot Soc Beng*, **11** (1957) 59-84.
12. Gangulee H C, Mosses of Eastern India, *Bull bot Soc Beng*, **13** (1959) 1-9.
13. Gangulee H C, Mosses of Eastern India, *Bull bot Soc Beng*, **14** (1960) 10-57.
14. Gangulee H C & Chatterjee N K, Cytological studies in the mosses of Eastern India. I, *J Indian bot Soc*, **39** (1960) 531-36.
15. Gangulee H C, Cytological studies in the mosses of Eastern India. II, *Nucleus*, **3** (1960) 165-76.
16. Gangulee H C, Cytological studies in the mosses of Eastern India. III, *Caryologia*, **15** (1962), 367-400.
17. Gangulee H C, Mosses of Eastern India. IV, *J Bombay natu Hist Soc*, **60** (1963) 606-37.
18. Gangulee H C, Additions to the mosses of Eastern India and adjacent regions. I, *Nova Hedwigia*, **7** (1964) 139-55.
19. Gangulee H C, Additions to the mosses of Eastern India. II, *Nova Hedwigia*, **12** (1966) 417-38.

20. Gangulee H C, Additions to the mosses of Eastern India and adjoining regions. III, *Bull bot Soc Beng*, **23** (1969) 131-34.
21. Dipti Singh & Gangulee H C, Cytological studies in the mosses of Eastern India. V, *Bull bot Soc Beng*, **23** (1969) 153-54.
22. Gangulee H C, The need of district records of Indian mosses and moss flora of some Eastern India districts, *Bull bot Soc Beng*, **24** (1970) 5-29.
23. Chatterjee A & Gangulee H C, Cytological studies in the mosses of Eastern India. VI, Karyotypes, *Nucleus*, **13** (1976) 118-25.
24. Gangulee H C, *Mosses of Eastern India and adjacent regions*, Vol 1 (1969-72), 1-830; Calcutta.
25. Gangulee H C, *Mosses of Eastern India and adjacent regions*, Vol. 2 (1974-77), 831-1546; Calcutta.
26. Gangulee H C, *Mosses of Eastern India and adjacent regions*, Vol. 3 (1978-80), 1547-2145; Calcutta.
27. Gangulee H C, *Handbook of Indian mosses* [American Publishing Co (P) Ltd, New Delhi] 1985, pp71.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

## P K Gupta

Gupta has made significant contributions in the area of plant cytogenetics and more recently in genetics and plant breeding. His work extending over a period of about 25 years has been concerned mainly with (1) cytogenetic studies and induced mutations in some cereals, millets, fodder grasses and pulses; (2) genetic analysis of yield traits in a number of crops using biometrical methods and development of breeding material in wheat and several legume crops; and (3) evolutionary studies in a number of genera from Gramineae, Leguminosae, and Compositae using multidisciplinary approach, with particular emphasis on structure and behaviour of chromosomes. This work was done with the help of a team of workers associated with him in various capacities.

Gupta's major contributions in cereal cytogenetics have been: (i) study of substituting ability leading to the establishment of homoeologous relationships of three rye (*Secale cereale*) chromosomes with those of wheat chromosomes using the technique of gametophytic compensation; (ii) production and study of about 50 intergeneric and interspecific hybrids involving the genera *Triticum* and *Hordeum*, leading to analysis of (a) genetic control of meiotic pairing in the polyploid species of the genus *Hordeum* and (b) analysis of the genetic system in the genus *Secale* affecting

meiotic pairing in intergeneric hybrids; (iii) reporting of an A-B translocation in *Secale cereale* and study of the effect of high temperature on chiasmata frequency in the presence/absence of B chromosomes; and (iv) reconstruction of new triticales (a new man-made crop) through chromosome manipulations and production of desirable strains for cultivation through wheat  $\times$  triticales crosses. The work on triticales also included a study of variation in heterochromatin content with the help of giemsa banding as well as by microdensitometry (DNA estimations).

Among pulses, cytogenetic studies were undertaken in six pulse crops, with emphasis on mungbean, chickpea and lentil. This involved the preparation of standard karyotypes in six pulse crops, production of colchipooids in mungbean, chickpea and lentil and synthesis of a translocation tester set in lentil. The basic work done included the study of variation in nuclear DNA content within and between the species.

In crop plants, particularly cereals (wheat and triticales) and pulses (mungbean, urdbean, chickpea), genetic analysis was conducted for yield traits. This involved the study of gene effects and various other parameters using different mating systems, and stability analysis using



different models available. The information generated through this study is of value in planning of breeding programmes in these crops. Consequently, breeding programmes were initiated in several crops leading to submission of entries for all-India trials in crops like wheat and mungbean. Breeding material in several other crops (triticale, pea, pigeonpea, chickpea and lentil) is in advanced stages of evaluation and should lead to the development of new varieties in the future.

Induced mutations were produced artificially in wheat, triticales, foxtail millet, mungbean, pea, chickpea, and lentils, leading to the identification of a number of interesting mutations of both academic and applied value. This led to the submission of four mungbean mutants for all-India coordinated trials. In pea, a mutant flowering about 25 days earlier than the parent variety T-163 was identified and is being utilized for breeding.

Among evolutionary studies, several genera of Gramineae (*Digitaria* and *Setaria*), Leguminosae (*Crotalaria*, *Indigofera*, *Medicago*, *Trigonella*, *Tephrosia*, *Trifolium*, *Melilotus*, etc.), and Compositae (*Helianthus*, *Zinnia*, *Chrysanthemum*, *Tagetes*) were studied adopting multidisciplinary approaches of cytology (chromosome numbers and karyotypes), chemotaxonomy (phenolics, free amino acids, etc.) and taximetry ( $D^2$  analysis), leading to hypotheses regarding the evolutionary trends in these genera. In several genera comprising some tropical fodder grasses (*Dichanthium*, *Bothriochloa*, *Heteropogon*, *Cenchrus*, *Paspalum*, etc.), the apomictic mode of reproduction and seasonal variation in the degree of apomixis were studied. Similarly, in the genus *Lolium* comprising important

temperate fodder grasses, a genetic assay of nuclear DNA in parents,  $F_1$  and  $F_2$  segregants of *L. rigidum*  $\times$  *L. temulentum* cross revealed that the 30% excess DNA found in one of the two species must have resulted from repetitive DNA; the structural basis of this DNA variation was examined through cytological study of the pachytene chromosomes in the  $F_1$  hybrids. This information on the mechanism of evolution will be useful for both basic and applied researches.

Gupta has edited jointly with Dr M S Swaminathan, FRS, the volume "Cytogenetics of crop plants" (Macmillan India Ltd., 1983), which is one of the best reference sources on the subject. Recently, he edited a volume, *Genetics and crop improvement* (1986), which includes proceedings of a symposium organized by him at Meerut in December 1984. Another series of volumes on *Crop cytogenetics* is under preparation in joint editorship with Prof. T Tsuchiya of Fort Collins, USA (Elsevier Science Publishers, The Netherlands). He has also authored two books, *Cytology*, *Genetics and Evolution and Genetics*.

### Selected Publications

1. Gupta P K, Studies on transmission of rye substitution gametes in common wheat, *Indian J Genet*, **29** (1969) 163-772.
2. Gupta P K, Homoeologous relationship between wheat and rye chromosomes: Present status, *Genetica*, **42** (1971) 199-213.
3. Gupta P K, Cytogenetic evolution in the Triticinae: Homoeologous relationships, *Genetica*, **43** (1972) 504-30.
4. Gupta P K, Transfer of disease resistance from alien species to wheat, *Proc 2nd Int. SABRAO Congress, 1973*, New Delhi, *Indian J Genet*, **34A** (1974) 491-500.
5. Gupta P K & Rees H, Tolerance of *Lolium* hybrids to quantitative variation in nuclear DNA, *Nature, Lond*, **257** (1975) 587-88.

6. Gupta P K, Nuclear DNA, nuclear area and nuclear dry mass in thirteen species of *Crotalaria* (Angiospermae, Leguminosae), *Chromosoma, Berl*, **54** (1976) 155-64.
7. Gupta P K, Evolutionary mechanisms in flowering plants: A multidisciplinary approach, *Nucleus*, **21** (1978) 117-24.
8. Gupta P K & Sharma N C, Synthesis and evaluations of triticales reconstructions at hexaploid level, *Proc 5th Int Wheat Genet Symp*, Feb 1978, New Delhi, 1978, 1179-85.
9. Gupta P K, Nuclear DNA and meiosis in parents,  $F_1$  hybrids and  $F_2$  segregants of a *Lolium temulentum*  $\times$  *L. rigidum* cross, *Nucleus*, **22** (1979) 177-81.
10. Gupta P K & Priyadarshan P M, Triticale—Present status and future prospects, *Adv Genet*, **21** (1982) 255-345.
11. Swaminathan M S & Gupta P K, Improvement of crop plants—Emerging possibilities, in *Cytogenetics of Crop Plants*, edited by M S Swaminathan, P K Gupta and U Sinha (Macmillan India, New Delhi), 1983, 1-18.
12. Gupta P K & Bahl J R, Cytogenetics and origin of some pulse crops, in *Cytogenetics of crop plants*, edited by M S Swaminathan, P K Gupta and U Sinha (Macmillan India, New Delhi) 1983, 405-40.
13. Gupta P K, Role of loss of heterochromatin in improvement of hexaploid triticales, *Nucleus*, **27** (1984) 100-7.
14. Gupta P K & Fedak G, Genetic control of meiotic chromosome pairing in polyploids in the genus *Hordeum*, *Can J Genet Cytol*, **27** (1985) 515-30.
15. Gupta P K & Baum B R, Nomenclature and related taxonomic issues in wheats, triticales and some of their wild relatives, *Taxon*, **35** (1986) in press.
16. Gupta P K & Fedak G, Inheritance of genetic variation in rye (*Secale cereale*) affecting homoeologous chromosome pairing in hybrids with bread wheat (*Triticum aestivum*), *Can J Genet Cytol*, **28** (1986) in press.



## S K Jain

The floras of several regions in India were studied by Jain. His field work over three decades in many unexplored and remote areas contributed to knowledge on floristics and vegetation of Rajasthan<sup>1</sup>, Gujarat, Maharashtra and northwestern and eastern Himalaya. He published about 50 papers on floristics and enriched the herbaria at Dehradun, Lucknow, Pune, Allahabad, Shillong and the National Herbarium at Calcutta. Living plants brought by him added to the holding of botanic gardens at these places.

As Director of the Botanical Survey of India, Jain organized and stimulated large scale botanical explorations and preparation of regional and local (district) floras; he initiated the preparation, editing and publication of the National Flora of India in 1978.

Jain studied the taxonomy of several tribes and genera, particularly in the families Poaceae<sup>2</sup> and Orchidaceae<sup>3</sup>. He published about 50 papers on the grasses of upper Gangetic plains<sup>4</sup>, Bengal, Bihar and Orissa, new taxa, and on revisions or synoptic taxonomic accounts of the tribes Garnotieae, Isachneae and Agrostideae, and the genera *Filipedium* Raizada & Jain, *Capillipedium* Stapf, *Eremopogon* Stapf, *Dipterygium* Decaisne, *Cynodon* Rich, *Oropetium* Trin., *Manisuris* L., *Arthraxon* Beauv., *Zornia* L., *Coix* L., *Anthoxanthum*

L., *Coelogyne* Lindl., *Enteropogon* Nees and *Hedychium* Koen. in India.

He described one new genus and about 20 new species and varieties, and discovered three genera and about 15 species and varieties for the first time in India. Jain also published on new names, new combinations, locations of types and other aspects of plant nomenclature, including *Paudhon ka Namkaran*, the Hindi adaptation of the International Code of Botanical Nomenclature.

Jain did pioneering work on the ethnobotany of central, eastern and north-eastern India, particularly in the states of Madhya Pradesh, Andhra, Bihar, Orissa and Assam, and published over 50 papers in Indian and foreign journals. The studies covered plants used in medicine<sup>5</sup>, as food<sup>6</sup>, fibres, dyes, gums, tannins, in adornment of body and dwellings, musical instruments, taboos, beliefs, sacred species, and origin of local names of plants among tribals. His studies brought to light some interesting and ingenious devices and gadgets for trapping game, fish, rats and other animals, for weighing and for warding off cattle from fields.

Jain's work established ethnobotany as an organized discipline in India, and many educational and research institutions created chairs, divisions and a special paper in MSc programmes on

---

Pitambar Pant National Environmental Fellow, National Botanical Research Institute, Lucknow-226001; Residence : A-26, Mall Avenue Colony, Lucknow.



ethnobotany. The symposia organized by him in India and abroad generated wide interest in this field. He edited the book *Glimpses of Indian Ethnobotany*<sup>7</sup>.

Jain became Founder-President of the Society of Ethnobotanists and Chairman of the Commission on Ethnobotany under the International Union of Anthropological and Ethnological Sciences.

Jain studied the phytogeography of India and published on Mt. Abu, Gujarat and Maharashtra. He undertook studies on endemism in Indian flora, an area which had not been given much attention since D Chatterji's work about half a century ago. Studies on Monocotyledons have shown about 35% endemism in India.

Jain also directed his interest to rare and threatened species and problems of conservation of habitats<sup>8</sup>. He wrote on rare plants, causes of threat, conservation measures and the role of botanic gardens in conservation. He was a pioneer in organizing several seminars on the subject and published a state-of-art report and a catalogue of threatened plants, and edited volumes on Conservation of tropical plant resources<sup>9</sup>, Assessment of threatened plants in India<sup>10</sup>, Botany of tiger habitats<sup>11</sup> and the first volume of Indian Plant Red Data Book<sup>12</sup>.

He organized intensive studies on the floristics of several national parks (e.g. Nanda Devi, Corbett, Dudhwa, Silent Valley—Wynaad) and wrote on the botany of Manas National Park. He organized the preparation of a Directory of Botanic Gardens, a Green Book dealing with rare plants under cultivation, and establishment of rare plant sections in gardens.

Based on his long experience in field botany, making and management of

herbaria and in writing and editing scientific material, he wrote the Hand Book of Field and Herbarium Methods<sup>13</sup>; it has been a handy guide for the collection and management of specimens, fundamentals of taxonomy, plant nomenclature and for the preparation of scientific manuscripts.

Jain also wrote and translated in Hindi. He translated Sporne's *Morphology of Gymnosperms*, Santapau's *Common trees*, his own book on *Medicinal Plants*<sup>14</sup> and numerous articles for *Bharat ki Sampada*. His Hindi version of ICBN has examples from Indian plants. Jain edited the book *Bharat ki Vanaspati*; it has 43 articles and is the first book in Hindi giving concise accounts of flora, vegetation, phytogeography, endemism, rare plants, ethnobotany, lower plants, some aspects of biosystematics and exotics in India.

In all, Jain has authored (or edited) about 20 books and 200 papers; these include some bibliographies<sup>15</sup>, reviews and obituaries. His contributions to floristics and taxonomy of grasses and orchids, medicinal plants, ethnobotany, endemism and rare or threatened species and habitats have been widely quoted and reviewed.

One genus and about 20 species of Indian flora are named after Jain (*Jainia*, *jainii*, *sudhanshui*).

Jain has undertaken to compile a synoptic account of Poaceae in India. It is necessary to prepare similar accounts of all major economic families of Indian flora. The remote and mountainous regions in India need more intensive botanising and must yield new taxa, new records and rediscovery of some presumably extinct species.

Ethnobotanical work has to proceed in three directions, namely, writing of treatises on selected plant groups (e.g. diseases-wise, use-wise), detailed accounts of some tribes (like Gonds, Mikirs) and general accounts of remote regions, e.g. in northeastern India.

Endemism in Indian flora needs to be studied and quantified further. There is urgent need for critical taxonomic work on selected genera and families of high endemism like Orchidaceae. The biology and status of threatened species have to be studied for more effective conservation and utilization.

### Selected Publications

1. Puri G S, Jain S K, Mukherjee S K, Sarup S & Kotwal N, Flora of Rajasthan, *Rec Bot Survey India*, **19** (1964) pp. 159.
2. Ved Prakash & Jain S K, *Poaceae—Tribe Isachneae*, in *Fasc Flora of India*, Vol 14 (Bot Survey India, Calcutta), 1984, 1-42.
3. Jain S K & Mehrotra A, *An inventory of Orchidaceae in India* (Bot Survey India, Calcutta), 1984, pp 133.
4. Raizada M B, Bharadwaja R C & Jain S K, Grasses of upper Gangetic plains. Pt I, *Indian For Rec Bot*, **4** (1961) 171-277; Pt II, *Indian For Rec Bot*, **6** (1964) 151-226.
5. Jain S K, Medicinal plant lore of the tribals of Bastar, *Econ Bot*, **19** (1965) 236-50.
6. Jain S K, Wild plant foods of the tribals of Bastar (Madhya Pradesh), *Proc natn Inst Sci India*, **30B** (1964) 56-80.
7. *Glimpses of Indian ethnobotany*, edited by S K Jain (Oxford & IBH, New Delhi), 1981, pp 365.
8. Jain S K & Sastry A R K, Safeguarding plant diversity in threatened natural habitats, in *Conservation of threatened natural habitats*, edited by A V Hill (Council for Scientific and Industrial Research, Pretoria) 1984, 155-64.
9. *Conservation of tropical plant resources*, edited by S K Jain and K L Mehra (Bot Survey India, Calcutta) 1983, pp 253.
10. *An assessment of threatened plants of India*, edited by S K Jain and R R Rao (Bot Survey India, Calcutta) 1983, pp 334.
11. *Botany of Tiger Habitats in India*, edited by S K Jain and A R K Sastry (Bot Survey India, Calcutta) 1983, pp 71.
12. *The Indian Plant Red Data Book I*, edited by S K Jain and A R K Sastry (Bot Survey India, Calcutta), 1984, pp 162.
13. Jain S K & Rao R R, *A hand book of field & herbarium methods* (Today & Tomorrow's Print & Publ, New Delhi) 1976, pp 157.
14. Jain S K, *Medicinal Plants* (National Book Trust of India, New Delhi) 1968 (4th Edn. 1983) pp 180.
15. Jain S K, Mudgal V, Banerjee D K, Guha A, Pal D C & Das D, *Bibliography of ethnobotany* (Bot Survey India, Calcutta), 1984, pp 157.



## B M Johri

After taking the BSc degree from Agra College in 1931, Johri continued further studies at the same college for MSc degree. During this period, he came in close contact with Prof. P Maheshwari, who had joined Agra College in 1929. Under his guidance, he worked on "Morphology and Embryology of Alismaceae and Butomaceae"; the DSc degree was awarded to him by the Agra University in 1936. Of special interest is Johri's discovery of pollen grains in the stylar canal and ovary of *Butomopsis lanceolata*. Prof. B Sahni regarded it as a relic of gymnospermy in a confirmed and unquestionable angiosperm. This finding received wide international attention. During 1937-47, he worked on the embryology of angiosperms at various places, but remained in constant touch with Prof. P Maheshwari who was then teaching at the University of Dacca.

At the invitation of Sir Maurice Gwyer, Vice-Chancellor of the University of Delhi, Prof. P Maheshwari joined as Head of the Department of Botany of this university in March 1949. Johri, who had joined this department in August 1948, was entrusted with the task of organizing a research school in the summer of 1949. The best he could do was to invite some college teachers—S Narayanaswami (Jaipur), B Tiagi (Aimer), H B Saxena (Gwalior) and B Singh (Agra). And so, after the

disbandment of the Maheshwari School at Agra in 1936, we started another school with a modest beginning. This, in due course, became one of the important international centres in reproductive plant biology—developmental, comparative, phylogenetic, and experimental. Even though Prof Maheshwari passed away in May 1966, the Department of Botany, University of Delhi, still bears his stamp.

In 1957, Johri worked with Prof. H E Street (Swansea, U K) and visited most of the important centres of research in tissue culture in Europe. Johri returned to India, with three problems in mind: (1) At Pavia (Italy), it had been shown that, without the host tissue, *Cuscuta* could not be grown in cultures, (2) at Louvain (Belgium), in the laboratory of Prof. P Martens, Dr Nast (USA) was experimenting with growing pollen grains and ovules (on a slide) to effect fertilization, and (3) Johri's own studies on *Ranunculus*, at Swansea, had shown that this is an excellent system for *in vitro* investigations. The researches at Delhi have shown that explants of total and semiparasites can be grown successfully in cultures without the host or the host extract. The success of *in vitro* pollination and fertilization and subsequent development of seed is considered to be another landmark. Investigations on *Ranunculus* have demonstrated that this is indeed a system par excellence for *in vitro*

---

Formerly, Professor of Botany, University of Delhi, Delhi-110007; Residence : Riviera Apartments, Flat 29N, 6th Floor, 45, The Mall Road, Delhi-110007.



experiments on nutritional requirements, and control of growth, development and differentiation.

Some highlights of other research work are presented below.

Hall (1902) reported that in *Limnocharis emarginata*, the zygote divides transversely into a suspensor cell and an embryonal cell. Only in those cases where polyembryony occurs does the suspensor cell undergo repeated divisions to form an embryogenic mass from which multiple embryos arise. Johri and Bhatnagar (1966) reinvestigated *L. emarginata* and concluded that Hall had probably examined a material wrongly identified as *L. emarginata*. Such a pattern of embryogenesis does not occur in any member of the Butomaceae, including *Limnocharis*.

Embryological investigations by Johri (1963) strongly support the earlier suggestion that the genus *Butomus* alone should be included in Butomaceae, and *Butomopsis*, *Hydrocleis* and *Limnocharis* should be transferred to Alismaceae.

Most of the species of *Cuscuta* examined embryologically have 3-celled pollen at shedding, and Polygonum type of embryo sac. In contrast, *C. reflexa*, studied by Johri and Tiagi (1952) showed 2-celled pollen as well as a small proportion of 3-celled grains at shedding time. The embryo sac conforms to the *Allium* type. Other interesting features of this taxon are: occurrence of two types of suspensor, absence of histogenic differentiation in proembryo, presence of a filiform and spirally-coiled embryo without cotyledons, and absence of a root cap. The suspensor may comprise several vesicular coenocytic cells and different

embryos show transitional stages so that in some embryos the suspensor comprises uninucleate cells and is highly reduced. This feature is not known in any other angiosperm. On the basis of embryological data, Johri and Tiagi support the separation of *Cuscuta* from the Convolvulaceae to a separate family, Cuscutaceae.

Maheshwari and Johri (1942) reported, in *Acalypha indica*, a rare type of tetrasporic embryo sac. Eight of the 16 nuclei arrange themselves into two polar and two lateral groups of two cells each. Only micropylar group differentiates into an egg and a synergid. The eight free nuclei fuse in the centre to form the secondary nucleus.

Among other embryological contributions made by Johri and his students, those relating to the Loranthaceae (Johri and Bhatnagar, 1972) may be considered as the most significant. The members of this family lack normal ovules; the embryo sacs differentiate in the ovary at the base of the stylar canal, or in the mamelon, which is a projection from the base of the stylar canal. Several embryo sacs develop concurrently in an ovary. In a number of taxa, at the 4-nucleate stage, tips of the embryo sacs elongate into the stylar canal and reach various heights. In *Helixanthera ligustrina*, the tip of the embryo sac sometimes grows as far as the stigmatic papillae. In *Moquiniella rubra*, 4-9 embryo sacs develop through the 42-48 mm long style and reach up to the base of the stigma. Subsequently, the tips of some of the embryo sacs curve backward for 2-4 mm. Such embryo sacs have not been reported in any other angiosperm.

Johri and Bhatnagar report that the development of endosperm in the

Loranthaceae is also exceptional. The primary endosperm nucleus of each embryo sac descends from the style to the lower part of the ovary and divides to form a cellular mass. The individual endosperms proliferate causing the intervening tissues to become obliterated; all the endosperms fuse to form a 'composite endosperm'.

In *Tapinostemma acaciae*, Johri and Prakash (1965) recorded the formation of finger-like processes from the basal part of endosperm. These processes invade the adjoining tissue, sometimes making their way deep into the thick-walled hypostase. This feature is also not met with in any other member of Loranthaceae.

Miers (1851) had suggested raising the subfamilies Loranchoideae and Viscoideae to the rank of families, but Danser (1929) did not favour this upgrading. The exhaustive data on the morphology and embryology of these two subfamilies, made available by P Maheshwari, Johri and their students, strongly support the elevation of the two subfamilies to the status of independent families. Calder and Bernhardt (1983) in "*The Biology of Mistletoes*" remark: "...it is therefore not surprising that the work of embryologists of the Delhi school made such a strong contribution to the debate on their status".

Johri and his students (see Johri and Bhatnagar, 1960) made a detailed study of the embryology and taxonomy of the Santalaceae.

In *Qunichamalium chilense*, the embryo sac extends beyond the ovule and comes to lie in the ovarian cavity. Johri and Agarwal observed that the synergid haustoria are exceptionally long and reach up to one-third the height of style, attaining a length of up to 1,200  $\mu\text{m}$  before

fertilization. The remnants of the haustoria persist up to the globular stage of proembryo. Synergid haustoria of such dimensions have not been reported in any other angiosperm.

The commercial cottons of the world belong to four species of *Gossypium*: *G. arboreum*, *G. barbadense*, *G. herbaceum* and *G. hirsutum*. Realizing that a detailed and connected account of the embryology and morphology of this economically important plant was lacking, in 1956, the Indian Central Cotton Committee (ICAR) sponsored a project on the "Embryology of Some Indian Cotton Species". During the ten-year period of the above project, under Johri's guidance, three research scholars (Joshi, Ramchandani, and Pundir) made a thorough investigation of the morphology, embryology (including some experimental aspects) and hybridization of *G. arboreum* and *G. hirsutum*, and received their doctoral degrees.

The cross *G. arboreum*  $\times$  *G. hirsutum*, which is highly desirable, does not yield viable seeds. The embryo attains the early dicotyledonous stage in 30% of the cross but aborts subsequently. The disintegration of endosperm 15 days after pollination has been suggested as the cause of failure of embryo development. J O Beasley's attempt to culture isolated young hybrid embryos did not meet with much success. Joshi and Pundir (1966) again attempted to culture the heart-shaped embryos resulting from the cross *G. arboreum*  $\times$  *G. hirsutum* and attained initial differentiation of cotyledons, a massive hypocotyl, and radicle. Pundir then cultured the ovules (containing zygote or 2-celled hybrid embryo), three days after pollination, on a medium containing myoinositol, and was able to



obtain viable seeds containing a massive embryo but without endosperm. In the absence of myoinositol, growth of the embryo remained arrested.

The other families whose embryology and morphology were worked out by Johri and his associates are: Aristolochiaceae, Berberidaceae, Boraginaceae, Gramineae, Hydrocharitaceae, Leguminosae, Limnathaceae, Moraceae, Rutaceae and Tamaricaceae.

Having reported the unusual features in the embryology and morphology of the Santalales, Johri became interested in the causal aspects of this interesting group of parasites.

The work of Johri and his students has demonstrated that the seeds of several parasitic Santalales can be germinated in culture in the absence of a natural host or its stimulus (see Johri and Bhojwani, 1971). The seeds of leafy mistletoes germinate on a rather simple nutrient medium. Interestingly, the *in vitro* seedlings of *Scurrula pulverulenta* and *Dendrophthoe falcata* have haustorial structures comparable to those formed in nature. Haustorial structures also differentiate from callused embryo. The *in vitro* differentiation of haustoria in these mistletoes, in the absence of a host, is unprecedented among the parasitic angiosperms.

A feature of special interest observed in the cultured embryos of *Amyema pendula*, by Johri and Bajaj (1964), was the activation of papillate outgrowths present at the radicular end of the embryo. In 10% of the cultures raised on WM (White's medium) + casein hydrolysate + IAA, the outgrowths formed embryoids which subsequently developed into shoots.

In its origin and nutritional role, the endosperm is of special significance among the angiosperms. In nature, the endosperm is subservient to the embryo and lacks the ability to differentiate organs. Interest in raising this tissue in aseptic culture had been shown as far back as 1930s. Until 1964, investigators had obtained merely tissue cultures with no signs of organ differentiation. This failure had prompted many investigators to give up using endosperm as a suitable material for morphogenetic work. It is this very challenge that prompted Johri to become interested in this system during early 1960s. His sustained interest and enthusiasm paid rich dividends and differentiation of triploid shoot buds from the mature cultured endosperm was achieved for the first time in *Exocarpus cupressiformis* (Johri and Bhojwani, 1965). Subsequently, three students of Johri (Bhojwani, Nag and Srivastava) undertook an in-depth study of the problem of morphogenesis in the cultured mature endosperm and totipotency of endosperm cells, and received their doctorate degrees.

Nag and Johri (1972) made detailed investigations on the influence of various physical and chemical factors in the formation of organs from the endosperm of some Santalales. It has generally been observed that a cytokinin by itself, or in combination with an auxin, is essential for shoot formation from endosperm. Experiments with *Taxillus vestitus* have shown that the embryo has an inhibitory effect on the formation of shoots from the endosperm. When the endosperm-halves are cultured, the buds first appear around the cut surface. The orientation of the endosperm-half on the medium seems to be important in determining the extent of differentiation of buds. If the medianly-split



endosperm is planted with the cut-surface in contact with the medium, 12-18 shoot buds appear (in 100% cultures) and if the cut surface is away from the medium, only 1-3 buds are formed (in 30% cultures)

There are evidences in literature which suggest a correlation between the loss of organogenic capacity of the callus in long-term cultures, and the change in chromosomal constitution of the cells. In the embryonal callus of *Nuytsia floribunda*, Nag and Johri (1969) noted a loss in the potentiality for root and shoot formation, while the chromosome number was maintained at the diploid level. In ageing cultures of the endosperm, embryo and other sporophytic tissues of *Dendrophthoe falcata*, *Taxillus cuneatus* and *T. vestitus*, the chromosome number was remarkably maintained at the initial ploidy level.

Johri and his students have extended the experimental approach to the interpretation of form and structure in angiosperms, especially through the techniques of tissue and organ culture.

While studying *in vitro* seed germination in *Allium cepa*, Johri, Guha and Maheshwari (1965) noted a striking response of the seedlings to auxins. On the basal medium, the seeds germinated, the cylindrical cotyledon emerged out of the seed, the radicle grew downward into the medium (positively geotropic), and established a root system. When the medium contained IAA, the roots curved upward in about 50% seedlings. This was followed by the development of 'aerial' roots and a small bulb. The ageotropic response of the seedlings was also caused by IBA, NAA and 2,4-D. TIBA enhanced the auxin effect instead of reversing it.

Whether this is necessarily a case of auxin-induced ageotropism or simply chemotropism needs to be confirmed, because when the culture tubes containing seeds sown on medium supplemented with  $10^{-6}$  M IAA were kept in an inverted position, the root-end showed positive geotropism like those on auxin-free medium.

Johri and Sehgal (1963, 1966) were able to grow to maturity, ovaries of *Anethum graveolens* (ovule containing zygote) and *Foeniculum vulgare* (ovule containing globular proembryo) on a simple nutrient agar medium. The *in vitro*-formed seeds germinated *in situ*. On a medium containing yeast extract, some of the seedlings produced floral buds which, however, failed to open. Besides normal development of the embryo, polyembryony due to budding or cleavage of the zygotic proembryo was quite common in both the species.

Johri has made a substantial contribution towards consolidating information by writing a number of critical reviews (singly as well as in joint authorship). The reviews on "*Physiology of Pollen*" (Johri and Vasil, 1961) and "*The Pollen and Pollen Tube*" (Johri and Vasil, 1960) have been read widely. Other subjects which Johri has covered through reviews are "*In Vitro Production of Embryos*", "*Female Gametophyte in Angiosperms*" and "*Embryology in Relation to Taxonomy*".

Another interest of Johri is the study of 'History of Biological Sciences' which he has taught for many years.

Johri has supervised and guided the research work of 24 research scholars on plant studies, and one on 'biology

education', for which PhD degree has been awarded by the University of Delhi. He has published 72 original research papers, 43 short papers, 40 seminar/symposium/conference papers, and a monograph on Loranthaceae (CSIR, 1972). He has published the following books: *Experimental Embryology of Vascular Plants* (edited; Springer, 1982); *Dictionary of Economic Plants in India* (jointly with U Singh and A M Wadhvani) (ICAR, 1983); *Embryology of Angiosperms* (edited; Springer, 1984); and *The Angiosperm Pollen: Structure and Function* (jointly with K R Shivanna) (Wiley Eastern, 1985). Another book, *Comparative Embryology of Angiosperms* (jointly with K B Ambegaokar) is due for publication by Springer in 1987. The last book in the series would be *Sexual Reproduction in Green Plants: An Evolutionary Approach*, due for publication in 1988. Johri edited the international journal *Phytomorphology* for over ten years.

Johri started teaching in 1931 and is still continuing to give invited lectures. He has travelled widely, attended numerous national and international conferences, occupied senior positions in several organizations, and is a Fellow/Member of several academic bodies.

Johri was awarded the Birbal Sahni Gold Medal by the Indian Botanical Society in 1970 for 'outstanding contributions to botany'. He was President of the Botany Section, Indian Science Congress Association, in 1971.

### Selected Publications

1. Johri B M, Studies in the family Alismaceae. 1. *Limnophyton obtusifolium* Mig. *J Indian bot Soc*, **14** (1935), 49-66.
2. Johri B M, The life history of *Butomopsis lanceolata* Kunth, *Proc Indian Acad Sci*, **4B** (1936) 128-38.
3. Sahni B & Johri B M, Pollen grains in the stylar canal and in ovary of an angiosperm, *Curr Sci*, **4** (1936) 587-89.
4. Johri B M & Garg S, Development of endosperm haustoria in some Leguminosae, *Phytomorphology*, **9** (1959) 34-46.
5. Johri B M & Vasil I K, Physiology of pollen, *Bot Rev*, **27** (1961) 325-81.
6. Johri B M & Bajaj Y P S, Growth responses of globular proembryos of *Dendrophthoe falcata* (L.f.) Ettings in culture, *Phytomorphology*, **15** (1965) 292-300.
7. Johri B M & Bhojwani S S, Growth responses of mature endosperm in culture, *Nature, Lond*, **208** (1965) 1345-47.
8. Joshi P C, Wadhvani A M (née Ramchandani S) & Johri B M, Morphological and embryological studies of *Gossypium* L., *Proc natn Inst Sci India*, **33B** (1967) 37-93.
9. Johri B M & Raj B, Morphological and embryological studies in the family Loranthaceae. 12. *Moquiniella rubra* (Spreng. f.) Balle. *Oest Bot Z*, **116** (1969) 475-85.
10. Johri B M & Bhojwani S S, Embryo morphogenesis in the stem parasite *Scurrula pulverulenta*, *Ann Bot*, **34** (1970) 685-90.
11. Johri B M, Differentiation in plant tissue cultures, *Presidential Address, Botany Section, 58th Indian Science Congress, Bangalore, Pt II* (1971) 159-86.
12. Bhojwani S S & Johri B M, Morphogenetic studies on cultured mature endosperm of *Croton bonplandianum*, *New Phytol*, **70** (1971) 763-68.
13. Johri B M & Srivastava P S, Morphogenesis in endosperm cultures, *Z Pflanzenphysiol*, **70** (1973) 285-305.
14. Nag K K & Johri B M, Cytology and morphogenesis of endosperm and embryo tissues of *Dendrophthoe* and *Taxillus*, *Cytologia*, **39** (1974) 801-13.
15. Nag K K & Johri B M, Experimental morphogenesis of the embryo of *Dendrophthoe*, *Taxillus* and *Nuytsia*, *Bot Gaz*, **137** (1976) 378-90.
16. Johri B M & Rao P S, Experimental embryology, in *Embryology of Angiosperms*, edited by B M Johri (Springer-Verlag, Heidelberg) 1984, 735-802.



## R N Kapil

Kapil has carried out extensive researches in plant morphology, anatomy, embryology and ultrastructure of the reproductive organs of flowering plants. His findings (observations as well as illustrations) have been cited and reproduced extensively in Indian, Russian and American books, authored and edited by scientists of international repute. He has published over 130 research and review papers.

Besides probing into a large number of embryologically unknown groups, such as the Cornaceae, Crossosomataceae, Frankeniaceae, Garryaceae, Hamamelidaceae, Haloragidaceae, Lemnaceae, Nyssaceae, Polemoniaceae, Schisadraceae and Zygophyllaceae, he has investigated several taxa of disputed systematic position (e.g., *Bischofia*, *Crossosoma*, *Daphniphyllum*, *Frankenia*, *Garrya*, *Pentaphragma*, *Philydrum*, *Nyctanthes*, *Schisandra*, *Theligonum*, etc.) and has helped in elucidating their taxonomic status and relationships. This voluminous work has played a significant role in establishing principles and practices in the use of embryology in taxonomic studies and has helped this discipline in gaining worldwide acceptance from systematic considerations.

Several new and interesting embryological phenomena have come to light as a result of Kapil's investigations; these include: (i) occurrence of

periplasmodial tapetum in *Acalypha indica*, (ii) Fritillaria type of embryo sac and nucellar polyembryony in *Euphorbia dulcis*, (iii) synergid polyembryony and ovules with twin nucelli in *Peganum*, (iv) fimbriate aril in *Crossosoma*, (v) bisporic embryo sac and protrusion of embryo sac beyond nucellar limits in *Camellia*, (vi) micropylar endosperm haustoria with papillate walls in *Frankenia*, (vii) unique type of Helobial endosperm in *Philydrum*, (viii) extra-micropylar embryo sacs and uninucleate micropylar endosperm haustorium in *Pentaphragma*, (ix) 2- or 3-celled chalazal endosperm haustorium in *Magnolia*, (x) massive suspensor haustorium in *Haloragis*, (xi) two types of embryo sac development in *Trollius*, (xii) five types of embryo sac development (mono-, bi- and tetrasporic) in a single species of *Delosperma*, (xiii) *ab initio* cellular endosperm in *Parrotiopsis*, (xiv) suspensor polyembryony in *Garrya*, (xv) peculiar placentation, consisting of one parietal and one axile in each ovarian locule, in *Bischofia*, and so on. A large number of plants (e.g., *Camellia*, *Chrozophora*, *Parrotiopsis*, *Tridax*) were reinvestigated and some earlier observations corrected.

In collaboration with the famous Russian embryologist, Prof. G. Gerassimova Navashina, Kapil examined the deleterious effects of low temperature on the process



of double fertilization in *Crepis capillaris*. His collaborative work with E H Newcomb at University of Wisconsin, Madison (USA), resulted in the discovery of new organelle-like structures termed microcylinders. He has also written several critical reviews in embryology in international journals, and these have not only updated our knowledge periodically but have also been instrumental in modernizing teaching and in popularizing new methods and fresh lines of investigation in this discipline.

Kapil initiated collaborative research programmes with Hugo de Vries Laboratory, Amsterdam (The Netherlands), on ovules and seeds, with special emphasis on seed appendages. At the Agriculture University, Wageningen (The Netherlands), work was carried out on the ultrastructure and histochemistry of antipodals and integumentary tapetum. In collaboration with Siena School in Italy, investigations were conducted on apomixis, transfer cells, microtubules in generative cells and ultrastructure of styles and stigmas.

In the field of plant anatomy, his outstanding contributions include those on *Mollugo cerviana* and *M. nudicaulis*; a comparative study of aerial and terrestrial roots of *Ficus benghalensis*; detailed analysis of morphological nature; mode of infection and senescence of root nodules in *Cajanus cajan*; and observation, for the first time, of anomocytic stomata on the inner epidermis of the wall of inferior ovary in *Corokia*.

Realizing the importance of periodical evaluation of the state of knowledge and with the aim of highlighting the avenues for future research, he has written some excellent reviews in the field of

embryology, which have helped in modernization of teaching and research in this discipline. He published "some recent examples of the value of embryology in taxonomy" to emphasize the increasing role of embryological information in systematics. In a chapter on "Ovule" in *Recent Advances in the Embryology of Angiosperms*, special attention was devoted to ovular appendages. Various aspects of biology and ultrastructure of embryo sac have been outlined in more recent publications. Some other reviews, such as "Endosperm in Euphorbiaceae—A critical appraisal" and "A fresh look at the process of double fertilization in angiosperms" offer examples of deep analysis and synthetic approach in embryology, which have been championed by him. He has been instrumental in popularizing new methods and fresh lines of investigation through his masterly expositions, such as "Contemporary spectacle in angiosperm embryology" and "Embryology and fluorescence microscopy—An assessment of integration", "Antipodals", "The integumentary tapetum", "Ultra-structure and biology of female gametophyte in flowering plants", "Central cell: Pre- and post-fertilization parameters" and "Some new pollen characteristics as revealed by SEM".

Kapil's current interests relate to palynology, ultrastructural and histochemical aspects of vegetative and reproductive organs as affected by diseases and pollutants. In the field of palynology, his work on the pollen flora of dominant plant communities and phytogeographically important plants from Western Himalayas is specially noteworthy, since it has played a significant role in reconstructing the

history of Quaternary vegetation, which, in turn, has immense application in knowing the age of the rocks. Apart from investigating several palynologically little known genera, such as *Hypericum*, *Crewia*, *Roylea*, *Bassia*, *Myrsine*, *Xylosma*, *Reinwardtia*, *Atylosia*, *Flacourtia*, *Cuscuta*, *Sterculia*, *Dodonaea*, etc. with the help of scanning electron microscope, Kapil and his students have examined the pollen of *Daphniphyllum* and *Coriaria* and elucidated their correct taxonomic status and relationships. Among other interesting results listed are the occurrence of: (i) dimorphic pollen in *Berberis* and *Leptodermis*; (ii) compound, spirally-sculptured viscin threads in *Oenothera*; (iii) superimposed reticulation in *Grewia*; (iv) spirally-sculptured columellae in *Flacourtia*; (v) spine-like excrescences on the dimorphic columellae of *Reinwardtia*; (vi) micropores on the elements of colpal membrane in *Bauhinia*; (vii) tetrad connections in *Caryopteris*; (viii) apertural cap in *Xylosma*; (ix) exinous bridges in *Rosa* and *Ocimum*; (x) smooth and branched viscin threads in *Rhododendron*; (xi) abnormal grains in *Hiptage*; (xii) a spiral sculpturing on the individual grains of compound pollen of *Acacia*; (xiii) sterile, linear tetrads in *Lantana*; (xiv) branching of columellae in the lumina of *Caryopteris*; and (xv) differences in the number of apertures in *Solanum*, *Vallaria*, *Carissa* and *Zizyphus*. Kapil is now concentrating on the study of pollen of Orchidaceae with the help of SEM, and has found several new and interesting features. He is guiding research on the histochemical and morphogenetic changes induced by insects and mites on the reproductive organs of *Salvadora* and *Terminalia*, and has reported several teratologies of the infected floral parts.

Kapil's deep interest in botanical research has led him into an untiring search for and analysis of ancient Indian scriptures and other works which have enabled a better appreciation of the indigenous status of biological sciences in India. Jointly with late Prof. P Maheshwari, he summarized "*A short history of botany in India*" and also published a book entitled "*Fifty Years of Science in India—Progress in Botany*". Subsequently, he wrote a detailed account of "*Biology in Ancient and Medieval India*" refuting an earlier notion (Burkill, 1965) that botany came to India only with British rule. He has published two important papers on "Ancient Indian system of nomenclature of plants" and "Portuguese contributions to Indian botany".

### Future Plan of Work

Kapil has ongoing research programmes on the (i) influence of pathogens (specially mites and insects) on the normal sex life of higher plants with the aim to evaluate the pathogen-host relationship, especially the morphogenetic behaviour of the reproductive parts as a result of infection, (ii) palynological studies of flowering plants, with emphasis on tropical orchids, with the scanning electron microscope; and (iii) effect of various pollutants on the biology of reproduction in higher plants. Simultaneously, ultrastructural and histochemical investigations on apomixis and hypostase are also being pursued.

### Selected Publications

1. Johri B M & Kapil R N, Contributions to the morphology and life history of *Acalypha indica* L., *Phytomorphology*, **3** (1953) 137-51.
2. Maheshwari P & Kapil R N, A short history of botany in India, *J Gauhati Univ*, **9** (1958) 1-34.



3. Maheshwari P & Kapil R N, *Fifty Years of Science in India—Progress of Botany*, Golden Jubilee Session, Indian Sci Congress, Delhi (1963) pp 178.
4. Maheshwari P & Kapil R N, Some Indian contributions to the embryology of angiosperms, *Phytomorphology*, **16** (1966) 239-91.
5. Masand, Pushpa & Kapil R N, Nutrition of the embryo sac and embryo—A morphological approach, *Phytomorphology*, **16** (1966) 158-75.
6. Gerassimova-Navashina Helen, Kapil R N, Korobova Stanislava & Savina Galina, The course of double fertilization at low temperatures (In Russian), *Bot Zh SSSR*, **53** (1968) 614-27.
7. Kapil R N, Contemporary spectacle in angiosperms embryology, *Acta bot Indica*, **2** (1974) 79-106.
8. Kapil R N & Bhatnagar A K, A fresh look at the process of double fertilization in angiosperms, *Phytomorphology*, **25** (1975) 334-68.
9. Kapil R N, Pugh T D & Newcomb E H, Microbodies and an anomalous "Microcylinder" in the ultrastructure of plants with crassulacean acid metabolism, *Planta*, **124** (1975) 231-44.
10. Kapil R N & Bhatnagar A K, Portuguese contributions to Indian botany, *ISIS, Wash*, **67** (1976) 449-52.
11. Kapil R N & Tiwari S C, Plant embryological investigations and fluorescence microscopy—An assessment of integration, *Int Rev Cytol*, **53** (1978) 291-31.
12. Kapil R N & Tiwari S C, The integumentary tapetum, *Bot Rev*, **44** (1978) 457-90.
13. Kapil R N & Bhatnagar A K, Ultrastructure and biology of female gametophyte in flowering plants, *Int Rev Cytol*, **70** (1981) 350-403.
14. Willemse M T M & Kapil R N, Antipodals of *Gasteria verrucosa* (Liliaceae)—An ultrastructural study, *Acta bot neerl*, **30** (1981) 25-32.
15. Ciampolini F, Cresti M & Kapil R N, Fine structural and cytochemical characteristics of style and stigma in olive, *Caryologia*, **36** (1983) 211-30.
16. Cresti M, Ciampolini F & Kapil R N, Ultrastructure of S<sub>3</sub>S<sub>4</sub> genotype pollen grains of *Oenothera organensis*, *Acta bot neerl*, **32** (1983) 177-83.
17. Cresti M, Ciampolini F & Kapil R N, Generative cells of some angiosperms with particular emphasis on their microtubules, *J submicrosc Cytol*, **16** (1983) 317-26.
18. Cresti M, Ciampolini F & Kapil R N, Further details on A and B type transfer cells from the leaves of *Linaria*, *J submicrosc Cytol*, **15** (1983) 767-72.
19. Kapil R N & Bhatnagar A K, Central cell : Pre- and post-fertilization parameters, *Phytomorphology*, **33** (1983) 157-67.
20. Sokhi Jaswant & Kapil R N, Morphogenetic changes induced by *Trioza* in flowers of *Terminalia arjuna*—I, Androecium, *Phytomorphology*, **34** (1984) 117-28.

## T N Khoshoo

Khoshoo's research career can be divided into six integrated phases, coinciding more or less with the positions he held as a teacher at the Panjab University, scientist at the National Botanical Research Institute (NBRI) (a CSIR laboratory), Director of NBRI, Secretary to the Government of India, and finally back to the CSIR system as Distinguished Scientist, a position personal to him. However, all through, particularly during his tenure at the University and at NBRI, his predominant research interest has been cytogenetics as related to plant evolution and improvement.

### Phase I (1948-55)

During this period, Khoshoo concentrated on the cytology and cytogenetic aspects of conifers (softwood timber trees); he later widened the scope of this work so as to cover the entire group of gymnosperms. On the basis of this work, he was able to unravel the genetic architecture of gymnosperms as a whole. He advanced new hypotheses to explain the different traits of the genetic system, as also the different mechanisms underlying evolutionary diversification in the group. This work has been of considerable practical importance in forest tree breeding programmes in chalking out breeding methodologies for this economically and environmentally

important group of plants. The basic idea has been to confer on these plants both immediate fitness and long-term genetic flexibility. In essence, the objective is to maintain a high degree of genetic diversity on forest tree populations, thereby simulating organic evolution, and ensuring survival under changing environmental conditions.

Khoshoo discovered, for the first time, in plant kingdom, an unique all-telocentric karyotype in the bizarre monotypic genus of gymnosperms, *Welwitschia mirabilis*.

Khoshoo, in association with D Ohri has recently turned his attention to the evolutionary implications of the variation in DNA content in karyotypes of gymnospermous taxa and the results of these investigations will be published shortly.

### Phase II (1955-64)

During this period, Khoshoo worked on experimental evolution in polymorphic weeds, and vegetable, minor fruit and medicinal plants. His work on the experimental analysis of variation and evolution within *Sisymbrium irio* complex is of special importance. This complex is an intricate mixture of diploid to octoploid races with rampant allo and autoallopolyploidy. He was able to synthesize experimentally the natural polyplotypes. Prof. G L



Stebbins has commented about this investigation as "a most excellent piece of work, truly exceptional in originality, initiative, the thoroughness of execution and entirely accurate. Any university in India or USA could consider itself proud to have a student capable of executing such a thesis." This work has been quoted in many books on the subject; one of these refers it as the "most elegant" work (*Genetics and Twentieth Century Darwinism*, Cold Spring Harbor Symposium Quantitative Biology, 1959).

### Phase III (1964-76)

This period marks the important phase of experimental analysis of both natural and domesticated variation, as also synthesis of new and improved cultivars of non-agricultural economic plants, particularly ornamental and subsidiary food plants. He was able to manipulate purposefully genes, chromosomes and genomes for increased novelty and/or productivity of some ornamental and subsidiary food plants. His major achievements in this area have been perfecting the breeding methodology for  $F_1$  hybrid seed production of triploid marigolds; free flowering colourful hybrid verbenas as ground cover; new tropicalized cultivars in amaranths, bougainvilleas, chrysanthemums, and gladiolus; tetra-giant snapdragons; transfer of resistance to grain amaranths from wild relatives for major pest (*Hypolixus trunculatus*), etc. Considering the fact that all over the world, improvement of ornamentals is essentially a nurseryman's trade secret, his aim was to build indigenous know-how in this area. Through this work, he has generated considerable new knowledge and good basic information has been unraveled

regarding the genetic-evolutionary race history of a number of such plants. Using cytogenetic techniques, he has worked out the genetic mechanisms underlying transformation of small and wild into large cultivated types during the process of domestication of ornamental and subsidiary food plants. Based on this knowledge, he also delineated, for the first time, centres of their diversity/origin, circumscribed gene pools and standardized procedures for studying the taxonomy of cultivated plants. Some of his cultivars in bougainvillea, amaranths, gladiolus and chrysanthemum have been regarded as unique and have gone in nursery trade throughout South East Asia and the tropical/sub-tropical areas. This work has earned him a place in ornamental horticulture for its high scientific quality on the one hand and for enabling enrichment of the beauty of environment and evolution of tropicalized cultivars on the other.

Thanks to the outstanding cytogenetic and biochemical work done by him and his team on protein-rich grain-amaranths with their carotene-rich leaves, today these crops, on account of their being short duration, hardy and less demanding, are high priority new crops of the world, particularly as sources of weaning food for mitigating protein malnutrition in the developing world.

### Phase IV (1976-82)

Although Khoshoo continued his work on non-agricultural economic plants during this period, his main preoccupation was the restructuring of R & D work of the National Botanical Research Institute (CSIR), Lucknow. He initiated high level research programmes, especially on pollution-tolerant plants for landscaping

polluted areas, aerobiology of allergenic plants, man-made forests and biomass production on marginal land using high-density and short rotation firewood, alcohol, petro and rubber yielding plants at the country's first Biomass Research Centre at Lucknow. This Centre has been considered unique in so far as plant-based energy systems are concerned. He also initiated work on standardization of herbal drugs and their compound formulations, particularly for rural use and development. The work on improvement of betelvine cultivation brought about the much needed modernization in this important but forgotten age-old industry affecting the lives of millions of subsistence farmers. The country's first integrated sewage grown *Spirulina* cultivation farm was established and its products like biogas, fertilizer, poultry feed, fisheries and irrigation water were put to use. Many of these projects have since spread, particularly those in the area of plants in relation to environment and rural energy. These changes in NBRI paid rich dividends and most botanical organizations have commended the dynamic approach which combines socio-economic relevance with scientific excellence. The impact of many of these programmes is apparent from the social, economic and environmental advantages that have accrued from them. In essence, the work during this period dealt with botany in relation to environment and energy. His Presidential Address entitled "Energy from Plants" at the Botany Section of the Indian Science Congress (1982) is regarded as a unique reference source, as it discusses, for the first time, the production, processing and utilization of biomass energy as relevant to developing countries.

Khoshoo's leadership in restructuring R and D plans of NBRI brought this institute

into the mainstream of the CSIR and at par with its major laboratories and resulted in elevation in 1978 of the then National Botanic Garden (NBG) to the level of a national institute which has now become a focal point of R & D on underutilized/non-traditional economic plants combining relevance with excellence.

### Phase V (1982-85)

During this period, in his capacity as Secretary, Department of Environment, Khoshoo was responsible for the formulation of policies and plans with regard to management of environment in the country, including the work of the Botanical and Zoological Surveys, National Museum of Natural History, the network organisation Himalayan Institute of Environment and Development, etc. The aspects covered included pollution monitoring and control, impact assessment, natural living resources conservation, eco-development, research promotion, education and training, information and publication, coordination and liaison with states, policy and law and international cooperation. The role of basic and applied sciences, engineering, socio-economic, law and community action was stressed. The Department of Environment was recognized as a systems builder, agent of change and a catalyst. Many innovations were brought in through All India Coordinated Programmes on heavy metal toxicity, rural fuels, fluorosis, air pollutants and plants, biotechnology of waste management, ethnobiology, tissue-culture and seed biology as a conservation strategy, etc. Meaningful programmes on eco-development of watersheds and ecologically degraded areas and environmental education, awareness, and training were initiated.



Centres of excellence on environmental research, training and education and mined areas development were identified and supported. All these and many other programmes like the Ganga Action Plan have brought together, in a symbiotic partnership, scientists, technologists, ex-servicemen, governmental and non-governmental organizations, students and villagers.

This period culminated in his General President's Address to the 73rd Session of the Indian Science Congress (1986) entitled "Environmental Priorities in India and Sustainable Development".

### Phase VI (1985 onwards)

Today, Khoshoo's principal interests at the R and D level are conservation of biological diversity, forestry for long-range ecological security and supply of goods and services to people and industry and utilization of derelict land for productive purposes. His interests at the policy research level are sustainable development and national conservation strategy.

### Selected Publications

1. Mehra P N & Khoshoo T N, Cytology of conifers, *J Genet*, **54** (1956) 165-85.
2. Khoshoo T N, Polyploidy in gymnosperms, *Evolution*, **13** (1959) 24-39.
3. Khoshoo T N, Cytogenetical evolution in the gymnosperms—Karyotype, *Proc Summer School*, Darjeeling, 1960, 119-35.
4. Khoshoo T N & Ahuja M R, The chromosomes and relationships of *Welwitschia mirabilis*, *Nature, Lond*, **193** (1962) 356-57; *Chromosoma*, **14** (1963) 522-23.
5. Khoshoo T N, Chromosome evolution in cycads, *Chromosomes Today*, **2** (1967) 236-40.
6. Khoshoo T N, Biosystematics of *Sisymbrium irio* complex, *Nature, Lond*, **176** (1955) 608.
7. Khoshoo T N, Biosystematics of *Sisymbrium irio* complex—IX: Genome analysis, *J Indian bot Soc*, **39** (1960) 217-26.
8. Pal M & Khoshoo T N, Grain amaranths, in *Studies in World Crops: Diversity and Change in Indian Subcontinent*, edited by J Hutchins (Cambridge Univ Press, London) 1974, 129-37.
9. Khoshoo T N, Cytogenetics in relation to plant evolution and deirnpovement, in *Progress in Plant Research*, Vol 2, edited by T N Khoshoo and PKK Nair (Today and Tomorrow's Printers and Publ, New Delhi) 1979, 1-74.
10. Khoshoo T N, Experimental taxonomy and its application to horticultural botany, *Bot Jahrb Syst*, **102** (1981) 97-117.
11. Khoshoo T N & Mukherjee I, Genetic-evolutionary studies on cultivated Cannas—IV: Origin and evolution of ornamental taxa, *Theor appl Genet*, **40** (1970) 204-17.
12. Raina S N & Khoshoo T N, Cytogenetics of tropical bulbous ornamentals—IX: Breeding systems in *Zephyranthes*, *Euphytica*, **21** (1970) 317-23.
13. Narain P & Khoshoo T N, Origin and evolution of garden amaryllis, *Indian J Hort*, **34** (1977) 80-85.
14. Zadoo S N, Roy R P & Khoshoo T N, Cytogenetics of cultivated bougainvilleas—VII: Origin and evolution of ornamental taxa, *Indian J Hort*, **33** (1976) 278-88.
15. Ohri D & Khoshoo T N, Cytogenetics of garden gladiolus—IV: Origin and evolution of ornamental taxa, *Proc Indian natn Sci Acad*, **49B** (1983) 279-94.
16. Khoshoo T N & Subrahmanyam G V, Cytogenetics of cultivated nymphaeas—IV: Origin and evolution of ornamental taxa, *Proc Indian natn Sci Acad*, under submission.
17. Khoshoo T N & Arora O P, Genesis of bivalent pairing in hexaploid clump *Verbena*, *Chromosoma*, **26** (1969) 259-69.
18. Khoshoo T N, Mehra R C & Bose K, Hybridity, polyploidy and change in breeding system in a *Ruellia* hybrid, *Theor appl Genet*, **39** (1969) 133-40.
19. Khoshoo T N & Pal M, Probable origin and relationships of garden cockscomb, *Bot J Linn Soc*, **66** (1973) 127-41.
20. Khoshoo T N, Energy from plants: Problems and prospects, *Presidential Address, Botany Section, Sixty-ninth Session, Indian Science Congress*, 1982, pp 112.
21. Khoshoo T N, Conservation of biological diversity: The Indian experience, *Proc US Strategy Conference on Biological Diversity*, 16-18 November 1981, 1982, 17-21.
22. Khoshoo T N, Perspectives in environmental

- management, *Keynote Address*, IIT, Bombay, 7 February 1983.
23. Khoshoo T N, Biosphere reserves: Areas for long-term conservation and management of biological diversity, *Presidential Address, Annual General Meeting, National Science Academy, India, Goa, 27 October 1983*.
  24. Khoshoo T N, Environmental Concerns and Strategies—*Collection of Lectures* (Indian Environmental Society, New Delhi) 1984, pp 296.
  25. Khoshoo T N, Environmental priorities in India and sustainable development, *General President's Address, Seventy-third Session, Indian Science Congress Association, 1986*, pp 224.



## B C Kundu

Kundu has been one of the foremost researchers in India and abroad in the field of jute. He did his MSc in botany from the University of Calcutta (1927) and obtained his PhD from the University of Leeds, where he took a special course in agricultural botany and soil science. He was Professor of Botany at the Presidency College, Calcutta till 1945. For the next 15 years (1946-60), he was Director, Jute Agricultural Research Institute, Calcutta. During this period, he was instrumental in the evolution of high yielding jute varieties like JRO-632 and JRO-753 (*olitorius*) and JRC-212 (*capsularis*), which are still considered important jute types throughout the jute growing areas. In addition, he evolved several high yielding kenaf (*mesta*) types which are being used in areas where jute cannot be grown successfully. He also worked on the utilization of the woody stalks of jute and mesta plants for the production of paper pulp. Kundu studied the fine structure of fibre and the role of leaf cuticle in preventing the attack of diseases and pests on jute plant. He also carried out taxonomic and anatomical studies on mesta and jute.

Kundu worked out for the first time the origin and development of jute and mesta fibres in the stem and the structure of jute fibres. He carried out intensive investigations on jute-paddy rotation, particularly for growing jute as an early

additional crop in paddy lands. He established the fact that row cropping of jute and kenaf is more profitable than broadcast sowing.

During 1965-70, Kundu worked as Joint Director and Research Professor at the Bose Institute, Calcutta. Currently, he is an honorary Research Professor at the Botanical Survey of India, Calcutta as also at the Jute Agricultural Research Institute, Barrackpore.

After the partition of India, in addition to his duties as Director of Research, Jute Agricultural Research Institute, he also worked as Director of Development and concentrated his efforts on jute and kenaf extension work and it was largely through his drive and initiative that India became self-sufficient in jute fibre production.

At the invitation of the US Department of Agriculture, Kundu attended the International Kenaf Conference held at Florida in 1964-65 and visited several research institutes and universities in USA. In 1966, at the invitation of the Peruvian Government, he attended the Agricultural Conference of Latin American countries held in Peru and Brazil and discussed important matters relating to the production of kenaf and jute. He was invited by the different agricultural research institutes in Brazil and Peru to discuss matters relating to research on fibre crops.

---

Formerly, Director, Jute Agricultural Research Institute; Residence : 332, Lake Town, Calcutta-700055.

In recognition of his researches in plant taxonomy, the Smithsonian Institution, Washington, DC, invited him to work in connection with the revision of the Flora of Ceylon; he recently completed the work after visiting Ceylon twice.

Through an invitation from the International Botanical Congress, he visited Seattle in 1969 and later visited several parts of USA regarding the utilization of kenaf fibres in the production of paper. He also received invitations from the Institute of Plant Industry, Leningrad, USSR, Fibre Research Institute, Taiwan and the European Institute of Fibres, Paris. In 1965 and again in 1969, he visited Tokyo and worked there in the University Botanical Garden for several weeks.

Kundu is 80 now and even at this age he is still active in the field and in the laboratory for further improving the yield and quality of jute.

Kundu was the President, Botany Section, Indian Science Congress Association, in 1954. He was President, Indian Botanical Society in 1958-60 and again in 1970. He has been Biological Secretary and later Vice President, Asiatic Society since 1976. He was awarded the Rafi Ahmed Kidwai prize and medal in recognition of his researches on jute. He has also won the Paul Johannes Brüh La medal of the Asiatic Society.

### Selected Publications

1. Kundu B C & Agharkar S P, Charophytes of Bengal, *J Dep Sci Calc Univ* (1937).
2. Kundu B C, New species and varieties of *Trichosanthes* from India, *J Bot, UK* (1939).
3. Kundu B C, Fine structure of phloem fibres—Untreated and swollen hemp, *Proc R Soc* (1940).
4. Kundu B C & Preston R D, Fine structure of phloem fibres, *Proc R Soc* (1941).
5. Kundu B C, On the multiperforate plates occurring in the xylem vessel of monocotyledonous roots, *Proc Indian Acad Sci* (1942).
6. Pal, B P & Kundu B C, Charophytes of India, *Indian Counc Agric Res, New Delhi* (1942).
7. Kundu B C, Anatomy of two Indian fibre plants—*Cannabis* and *Corchorus*—with special reference to the growth and development of fibres, *J Indian bot Soc*, **21** (1942) 93-128.
8. Kundu B C, Anatomy of jute stem with special reference to cambial activities and distribution of fibres in relation to leaf trace system, *J R Asiat Soc Beng*, **10** (1944) 27-52.
9. Kundu B C, Differentiation of vascular tissues in *Hibiscus subdariffa*, *J Indian bot Soc* (1944).
10. Kundu B C, The effect of X-irradiation on the growth and development of jute plants, *Proc natn Inst Sci India*, **12** (1966) 181-85.
11. Kundu B C, Ghosh R L M & Rao K R, Genetics of corchorus—Inheritance and linkage relation to bitter taste, anther and corolla colour, *J Genet*, **49** (1948) 14-22.
12. Kundu B C & Rao N S, Effect of gammexane on the root tips of *Corchorus capsularis* Linn, *Sci & Cult*, **14** (1949) 484.
13. Kundu B C, Origin of jute, *Indian J Genet Pl Breed*, **11** (1951) 95-99.
14. Kundu B C & Srinath K V, Cytological studies of pollen tube growth in reciprocal crosses between *C. capsularis* and *C. olitorius* L, *Cytologia, Japan*, **17** (1952) 219-23.
15. Kundu B C & Rao N S, Origin and development of axillary buds in jute (*Corchorus capsularis*), *Nature, Lond*, **130** (1952) 1128.
16. Kundu B C, Origin, development and structure of important vegetable fibres, *Presidential Address, Botany Section, Indian Science Congress, 41st Session, Hyderabad*, 1954.
17. Kundu B C & Rao N S, Origin and development of axillary buds in jute, *Ann Bot, Lond, New Ser*, **28** (1954) 367-75.
18. Kundu B C & Rao N S, Effects of maturity on the dimensions of ultimate fibres of jute, *J scient ind Res*, **14B** (1955) 124-27.
19. Kundu B C & Rao N S, Origin and development of axillary buds in *Hibiscus cannabinus*, *Am J Bot*, **42** (1955) 830-37.
20. Kundu B C & Sharma M S, Studies on colchicine-induced tetraploids of *Corchorus olitorius* Linn. (jute), *J Indian bot Soc*, **33** (1956) 11-26.



21. Kundu B C, Jute, world's most important bast fibre crop (Botany, agronomy, diseases and pests), *Econ Bot*, **10**(2) (1956) 102-6.
22. Kundu B C, Jute, world's most important bast fibre crop (Technology, marketing, production and utilisation), *Econ Bot*, **10**(3) (1956) 203-40.
23. Kundu B C, Dutta A N & Sanyal P, Comparative study of yield performance of different fibre crops, *Indian J Agron*, **3** (1959) 231-36.
24. Kundu B C, *Jute in India : Agriculture* (Indian Central Jute Committee, Calcutta) 1939.
25. Kundu B C, Mesta in India, In *Proc 2nd Int Kenaf Conf*, Palm Beach, Florida (1964) 249-63.
26. Kundu B C, Sun-hemp in India, *Soil Crop Sci Soc, Florida Proc*, **24** (1964) 396-404.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

## R N Lakhanpal

Although the known records of Tertiary plants in India go back to the later part of the eighteenth century, they remained practically uninvestigated until the first quarter of the present century. Their study really started in the early thirties, but was mostly confined to the description of stray fossils, more by way of recording their occurrence in different horizons. This practice was still continuing when Lakhanpal took up research on Tertiary plants under the guidance of Prof. B Sahni in 1945.

Lakhanpal started with the investigation of microfossils recovered from the saline Series of Punjab Salt Range (now in Pakistan) and provided evidence in favour of the view that the Saline Series was of Early Tertiary age. Soon after, he described some leaves and fruits of *Guttiferae* from the Eocene deposits of Western Rajasthan, a petrified palm from the Deccan Intertrappean beds, and some Tertiary plant remains from the Garo Hills, Eastern India. Among the Garo Hills fossils was a fruit of *Nypa*<sup>1</sup>, which at that time was the first report of this genus from the Miocene, all others till then having come from the Eocene beds. It indicated that the coastline of the Bay of Bengal extended as far north as the Garo Hills during the Miocene epoch.

From early 1952 to August 1953, he was a UNESCO scholar in palaeobotany and

spent most of his time at the University of California, Berkeley, specializing in Tertiary Palaeobotany under the foremost authority on the subject at that time, Prof. R W Chaney. In order to acquire proficiency in all the aspects of this branch, he carried out extensive field work and studied an Oligocene flora from a locality called Rujada<sup>2</sup> in Oregon, USA. During this study, Lakhanpal realized that the right approach to the investigation of Tertiary plant remains was not to describe individual taxa from different localities in a haphazard way but to treat the specimens from the same locality as members of a flora that grew together and to draw from their collective evidence the ecological and phytogeographical inferences to build up the historical geology of the region. Such an approach provided some meaning and objective to the study of Tertiary plants instead of describing them monotonously as mere additions to information. Considering the rich deposits of Tertiary strata in the Indian subcontinent and the enormous scope of the study of their plant remains, he decided to return home to devote himself to the establishment of Tertiary palaeobotany in India.

For a few years since 1954, he along with his research associates, concentrated his attention on the petrified flora of the Deccan Intertrappean Series in Central India and the Cuddalore Series of South

---

Emeritus Scientist, Birbal Sahni Institute of Palaeobotany, Lucknow-226007; Residence : B-21, Sector A, Mahanagar, Lucknow-226006.



India, discovering many new taxa and revising some of the previously described fossils. All these studies were ultimately put together in the form of a review paper on the Tertiary floras of the Deccan Trap Country<sup>3</sup>.

In the middle 1950's, Lakhanpal also initiated research in Recent and Quaternary palynology at the Birbal Sahni Institute and some of his early students have developed into well known palynologists. A survey of the atmospheric pollen at Lucknow<sup>4</sup>, carried out jointly with P K K Nair, is regarded as the beginning of modern aerobiology in India. In another paper<sup>5</sup> entitled "Palynology and Taxonomy", he convincingly brought out the fact that although not universally applicable, palynology does provide an important evidence in plant taxonomy. Therefore, it was suggested in those initial years in the history of palynology, that detailed studies of pollen and spore characters should be undertaken in all the plant groups. Besides, he published another two or three papers in palynology. However, this early association with palynology was only a brief interlude in his continued efforts at promoting Indian Tertiary palaeobotany.

With the growing interest of many Indian workers in this discipline, it was considered appropriate to take stock of the then existing situation and to chalk out future lines of investigation. Consequently, a state of art report<sup>6</sup> entitled "The present position and problems of Tertiary palaeobotany in India" was published by him in 1966.

In the 1960's, he extended his studies from Central and Southern India to the Tertiary plants of the other regions of India also, especially the Himalayan foothills. At

the same time, he also investigated some plant remains from the Middle Tertiary of Congo (Zaire)<sup>7</sup>.

Based on his extensive and critical observations, Lakhanpal prepared an in-depth review of the Tertiary floras of India<sup>8</sup>, which forms the first detailed consideration of the Palaeotropical Tertiary geoflora. It was presented as an invited paper at an All Congress Symposium in the Eleventh International Botanical Congress in 1969. He established the differentiation of Tertiary floras of India into two main groups of Palaeogene and Neogene. Based on all the palaeobotanical and related geological information, the palaeogeography of India during the Lower Eocene and Miocene times was reconstructed in the form of two maps. Similarly, ecological conditions prevailing in different regions of the country during the different epochs of the Tertiary period were also reconstructed from the evidence of fossil floras occurring there. Another significant conclusion that emerged was that in the tropics, water has been the main factor controlling the distribution of Tertiary plants in contrast to temperature which is the effective factor in temperate regions.

Phytogeographically, Dipterocarpaceae is one of the prominent families of tropical angiosperms. Along with the information regarding its modern distribution, a sufficiently reliable record of dipterocarpaceous fossils is available over a large area. Giving the details of these data, the possible centre of origin and distribution of this interesting family through geological time were discussed in a contribution<sup>9</sup> to a symposium convened by Lakhanpal on the origin and phytogeography of angiosperms.

One of the most baffling problems of angiosperm evolution is their origin. There are, no doubt, palaeobotanical records with a bearing on this problem, but opinions differ about their being true angiosperms. This aspect of the origin of angiosperms in the light of palaeobotanical evidence was discussed at length by him in a paper<sup>10</sup> titled "The antiquity of angiosperms". Unfortunately, the early history of angiosperms is still shrouded in mystery, as there are no unquestionable records in any period before the Cretaceous.

Going through the literature, one cannot help noticing the diversity that exists in the nomenclature of fossil dicot woods. Usually, the new names are coined by adding the ending *inium* or *oxylon* to the name of a recent genus or family. But new names are also formed after the names of persons, localities, rock formations, etc. For the sake of uniformity and to avoid any ambiguous affinity with a modern taxon, Lakhanpal, in conjunction with a specialist in Tertiary Xylotomy, made some useful suggestions regarding the nomenclature of fossil dicotyledonous woods<sup>11</sup>.

In the mid 1970's, Lakhanpal and two of his associates undertook the study of Tertiary plant remains from Kachchh in Gujarat and described them in a number of papers. Finally, a consolidated and critical account, embracing all the known records, was published in 1984 as Part III of a monograph on the fossil floras of Kachchh<sup>12</sup>. In general, the Tertiary plants of Kachchh indicate a moist tropical climate in which there was an admixture of evergreen to deciduous elements. The climate of Kachchh obviously deteriorated

to its present day dry conditions during the post-Tertiary period.

Lately, he has taken up the investigation of Tertiary plants from Ladakh. A specimen of a truly palmate palm collected by a party of the Geological Survey of India from the Liyan Formation (Miocene) of this area was identified as a leaf of *Trachycarpus*<sup>13</sup>, a temperate palm. This is the first definite megafossil record of a member of the temperate element which seems to have entered the Himalayas during the Miocene from the mainland of Asia on the north. In this publication, it was pointed out that some other temperate plants must also have accompanied *Trachycarpus*, for which a thorough search in the Liyan Formation of Ladakh was suggested.

Working in the Ladakh area, a party of the Wadia Institute of Himalayan Geology collected a fairly well preserved large palm leaf from the Hemis Conglomerate horizon which can be considered to be Eocene or early Oligocene. This palm was identified as *Livistona*<sup>14</sup>, a tropical palm. Its occurrence indicates that the climate of Ladakh was tropical during the Eocene-Early Oligocene time. Obviously, till then the Himalayas had not risen high enough for the temperate element to thrive there. It was the upthrust during the Miocene that brought about conditions suitable for the growth of temperate element.

Another party of the Wadia Institute collected a specimen of petrified wood from the Kargil Formation of Ladakh, an equivalent of Liyan Formation from which *Trachycarpus* was described. This wood was found to belong to the genus *Prunus*<sup>15</sup>, showing close resemblance with



the species *Prunus cerasoides*, which is a temperate plant growing on the southern flanks of the Himalayas. Its occurrence vindicates the view that the temperate element entered into the Himalayan flora during the Miocene, as first suggested on the find of *Trachycarpus* in the Liyan Formation of Ladakh.

The above-mentioned recent developments in the identification of Tertiary plants and their palaeoecological interpretation indicate that Tertiary palaeobotany in India, initiated about three decades ago, has come of age. It is time that besides studying new material, a critical revision is made of all the Tertiary plants recorded so far from the different localities of India. This would ensure correct listing of all the floras, on the basis of which dependable palaeoecological and phytogeographical conclusions could be drawn. Attempts should also be made to identify the palynological fossils in terms of their natural affinities, so as to provide corroborative evidence to strengthen the inferences based on megafossils. With these basic data in order, it would be possible to reconstruct the various Tertiary floras growing in different basins of our subcontinent and evolving according to the ever-changing physical conditions of their environments.

### Selected Publications

1. Lakhanpal R N, *Nipa Sahnii*, a palm fruit in the Tertiary of Assam, *Palaeobotanist (Birbal Sahni Memorial Volume)*, 1 (1952) 289-94.

2. Lakhanpal R N, The Rujada flora of West Central Oregon, *Univ Calif Publ geol Sci*, 35(1) (1958) 1-66.
3. Lakhanpal R N, Tertiary floras of the Deccan Trap Country, *Bull Indian natn Sci Acad*, No 45 (1973) 127-55.
4. Lakhanpal R N & Nair P K K, Survey of the atmospheric pollen at Lucknow, *J scient ind Res*, 17C (1958) 80-87.
5. Lakhanpal R N, Palynology and taxonomy, *Mem Indian bot Soc* No 1 (1958) 19-23.
6. Lakhanpal R N, The present position and problems of Tertiary palaeobotany in India, *Palaeobotanist*, 14(1-3) (1965); (1966) 202-8.
7. Lakhanpal R N, Some Middle Tertiary plant remains from South Kivu, Congo, *Ann Mus R Afr Centre sci Geol*, 8(52) (1966) 19-39.
8. Lakhanpal R N, Tertiary floras of India and their bearing on the historical geology of the region, *Taxon*, 19(5) (1970) 675-94.
9. Lakhanpal R N, Geological history of the Dipterocarpaceae, *B Sahni Inst Palaeobot Spl Publ* No 1 (1974) 30-39.
10. Lakhanpal R N, The antiquity of angiosperms, *Asp Pl Sci*, 1 (1976) 41-52.
11. Lakhanpal R N & Prakash U, Suggestions regarding the nomenclature of fossil dicotyledonous woods in *Modern Trends in Plant Taxonomy-5. Glimpses in Plant Research* (Vikas Publishing House, New Delhi) 1980, 199-208.
12. Lakhanpal R N, Guleria J S & Awasthi N, The fossil floras of Kachchh. III-Tertiary megafossils, *Palaeobotanist*, 33 (1984) 288-319.
13. Lakhanpal R N, Gyan Prakash, Thussu J L & Guleria J S, A fossil fan palm from the Liyan Formation of Ladakh (Jammu and Kashmir), *Palaeobotanist*, 31(3) (1984) 201-7.
14. Lakhanpal R N, Sah S C D, Sharma K K & Guleria J S, Occurrence of *Livistona* in the Hemis Conglomerate Horizon of Ladakh, in *Geology of Indus Suture Zone of Ladakh* (Wadia Institute of Himalayan Geology, Dehra Dun) 1983, 179-85.
15. Guleria J S, Thakur V C, Viridi N S & Lakhanpal R N, A fossil wood of *Prunus* from the Kargil (=Liyan Formation) of Ladakh, in *Geology of Indus Suture Zone of Ladakh* (Wadia Institute of Himalayan Geology, Dehra Dun) 1983, 187-93.

## C P Malik

Significant contributions have been made by Malik in the field of Developmental Physiology of Higher Plants, especially in relation to reproductive systems. Different experimental techniques have been adopted to collate structure and function at the cellular level. More recent work pertains to the regulation of crop yield by growth regulating chemicals. Some of the salient contributions are discussed below in brief.

### **(1) Non-photosynthetic Effects of Carbon Dioxide in the Context of Plant Developmental Systems**

(a) *Pollen tube growth* : Supplementing pollen suspension cultures with elevated levels of CO<sub>2</sub> were found to stimulate pollen germination and tube growth. The stimulatory effect of CO<sub>2</sub> is mediated partly via non-photosynthetic fixation of the gas predominantly by PEP carboxylase as revealed by <sup>14</sup>C-labelling kinetic patterns and complementary enzymic studies. It was concluded that the ability to assimilate CO<sub>2</sub> by germinating pollen and synthesize respiratory substrates, amino acids and proteins, osmoregulatory solutes, membrane lipids and NADPH, etc., appears to be an important growth mechanism in the plant reproductive cycle.

Besides PEP carboxylase, the contribution of other carboxylating enzymes is being examined. Additionally,

regulation of non-photosynthetic metabolism of CO<sub>2</sub>; role of PEP carboxylation in determining the rates of NH<sub>4</sub><sup>+</sup> assimilation; and effect of growth substances on non-photosynthetic CO<sub>2</sub> fixation are topics for future research in our laboratory.

(b) *Pollen-stigma interaction* : Elevated levels of CO<sub>2</sub> were shown to overcome sporophytic self-incompatibility response of Brassica species. CO<sub>2</sub> *per se* was shown to affect (i) pollen-pistil recognition by causing changes in the primary binding stage, (ii) pollen hydration, (iii) release of esterases, (iv) tube penetration into stigmatic papilla wall, and (v) induction and/or activation of callase ( $\beta$ ,1,3-glucanase) activity. In addition, the incorporation of non-photosynthetically fixed <sup>14</sup>CO<sub>2</sub> into several organic and amino acids was found to be an essential component for full expression of self-incompatibility.

Our future emphasis is on investigating direct effects of CO<sub>2</sub> in the proteinaceous pellicle seeking clues to the events occurring prior to pollen germination, i.e., how high CO<sub>2</sub> level affects the interaction of stigma surface receptors with the primary signal to check the induction of callose synthesis. The role of CO<sub>2</sub> in increasing the flow of water from papillae to pollen by affecting membrane lipids



appears feasible. The effect of  $\text{CO}_2$  on the hydrodynamics of pollen is another fascinating area of interest.

(c) *Embryogenesis* : Non-photosynthetic PEP carboxylation was shown to be an integral part of embryo growth in *Tropaeolum*. Further, a role of suspensor in the synthesis, storage and transport of several metabolites, including malate, to the developing embryo for osmoregulation and metabolism was demonstrated.

(d) *Cotton fibre development* : Developing cotton fibres were found to possess an active system for non-photosynthetic assimilation of  $\text{CO}_2$ . The parallel behaviour of  $\text{K}^+$  and non-photosynthetically derived malate during fibre elongation suggested their interrelationship as counteracting osmolytes. The levels of  $\text{K}^+$  and malate with respect to each other were shown to be important in determining the extent of fibre growth in cottons with different fibre lengths. The synthesis of malate was shown to be the main route of metabolism of fixed carbon during the period of rapid fibre extension, chiefly because of the requirements of osmoregulation. During the slowing down of the growth rate concomitant with the onset of secondary wall synthesis in fibres, the accumulated malate is degraded to support respiratory activities of the fibres and provide NADPH.

(e) The roles of non-photosynthetic PEP carboxylation in relation to nitrogen assimilation of maize seedlings have been studied. Purification of PEP carboxylase has been made and its kinetics studied.

## A Synthesis

Our studies show that non-photosynthetic carboxylation reactions are important in linking major biochemical

pathways. Sufficient evidence regarding PEP branch-point, and its importance in governing entry into and exit out of the TCA cycle is provided. Our studies on several developing systems have emphasized the necessity for revising concepts about the energetics and regulation of glycolysis in plants. Evidence provided by me points out that PEP carboxylase reaction may be more concerned with the production of intermediary metabolites than with simply supporting the TCA cycle.

Almost every plant process spanning from seed development and germination to flowering and fruit-set requires the essential participation of non-photosynthetic carbon fixation in regulating certain metabolic and cellular functions. An appreciation and clear understanding of this process is of vital importance for future research thrusts in  $\text{CO}_2$  metabolism studies.

Some of the areas which merit attention are mentioned below :

—Details of the control of PEP metabolism via PEP carboxylase and pyruvate kinase.

—Isozyme separation and purification of multiple forms of PEP carboxylase in order to compare their physicochemical properties.

—Effects of stress on non-photosynthetic fixation.

## (2) Reproductive Physiology of Higher Plants

(a) *Physiology of germinating pollen* : In the area of pollen physiology, the major contributions made include:

—Discovery of dark metabolism of CO<sub>2</sub> in germinating pollen.

—Hormonal regulation of pollen germination and tube growth.

—Cyclic AMP regulation of enzymes and lipid biosynthesis during tube growth.

—Phytochrome regulation of pollen tube metabolism.

—Evaluation of the role of glycosidases in pollen tube growth.

—Elucidation of the role of boron in pollen germination and tube growth. Boron was shown to play a role in partitioning of carbon flux between glycolytic and pentose phosphate pathways.

Efforts are now underway to employ pollen as a biotechnological tool for crop improvement.

(b) *Developmental physiology of female gametophyte*: Using histochemical and cytochemical techniques, the distribution of several metabolites, the precise pathway of the localization of the metabolites to the areas of utilization during various stages of megasporogenesis, megagametogenesis and embryo sac differentiation has been established. A correlation between enzymatic changes and structural changes, operative during active and quiescent stages of female gametophyte differentiation, has been shown. For the first time, the role of antipodals in the storage, supply and synthesis of certain metabolites was demonstrated. Further, the role of phenolic compounds and different growth substances in the differentiation of female gametophyte has been modulated.

(c) *Physiological characteristics of suspensor*: Histochemical and biochemi-

cal techniques were used to characterize specific physiological functions of the suspensor in early embryogenesis of *Tropaeolum* and *Phaseolus*. Among the functions established are absorption, translocation and biosynthesis of metabolites, as well as regulation of embryo differentiation. Controlled basipetal autolysis of suspensor by hydrolytic enzymes during late embryogenesis was shown against the prevalent view of degeneration of suspensor caused by expanding embryo.

It is important that comparative investigations should be carried out on as many divergent systems as possible. The precise physiological role of basal cell mass, apical cell rosette, in *Tropaeolum* is being established. How is the absorption of different macromolecules from the *Tropaeolum* fruit wall brought about by the carpel and placental haustorium? How do the hydrolytic enzymes affect such an absorption? Attention is also given to the hydrolases, whether excreted or released.

### (3) Physiology of Crop Yield

(a) Some of the low-priced phenolic acids (1,2,4-acid, H-acid and resorcinol) have been shown to increase yield in groundnut, moong, rice and winter maize. The phenolic acids also increased the quality attributes of lipids in groundnut.

(b) Foliar application of polyols (Mixtalal) was shown to enhance the yield of rice by suppressing photorespiration. Mixtalol or sodium benzoate used as a seed-soak in pigeon pea enhances yield and also provides resistance to the seedlings against moisture stress.

(c) My basic research findings led to the adoption of IAA and ethrel sprays for increasing the productivity of groundnut



(M-13); it is included in the 'Package of Practices for Kharif Crops, 1982' recommended by Punjab Agricultural University, Ludhiana. Application of the growth regulator 'Atonik' enhanced the free fatty acid contents in groundnut.

### Selected Publications

1. Malik C P & Vermani S, Physiology of sexual reproduction. II. A histochemical study of embryo sac development in *Zephyranthes rosea* and *Lagenaria vulgaris*, *Acta Histochem*, **51** (1975) 244-80.
2. Malik C P, Female gametophyte in angiosperms: Recent advances in histochemistry, ultrastructure and physiology, in *Glimpses in Plant Research*, III, edited by P K K Nair (Vikas Publishing House, Delhi) 1976, 114-47.
3. Malik C P & Singh M B, Fluctuations in the dehydrogenases activity during the development of pollen tubes, *Biochem Physiol Pflanzen*, **169** (1975) 583-89.
4. Singh M B, Malik C P & Sood R, Soluble and insoluble invertases in the pollen grains of *Pinus roxburghii*, *Z Pflanzen Physiol*, **81** (1977) 184-88.
5. Singh M B, Malik C P & Thapar N, Changes in the activities of some enzymes of carbohydrate metabolism in *Amaryllis vittata* pollen suspension cultures, *Pl Cell Physiol*, **19** (1978) 677-84.
6. Chhabra N & Malik C P, Influence of spectral quality of light on pollen tube elongation in *Arachis hypogaea*, *Ann Bot*, **42** (1978) 1109-17.
7. Kapur A, Malik C P & Dhawan A K, Nitrate assimilation in *Crotalaria juncea* Linn. Pollen suspension culture, *Pl Cell Physiol*, **19** (1978) 685-89.
8. Bhandal I S & Malik C P, Effect of gibberellic acid, phosphonic acid, actinomycin-D and cycloheximide on the activity and leaching of some hydrolases in pollen suspension cultures of *Crotalaria juncea*, *Physiol Pl*, **45** (1979) 297-300.
9. Dhawan A K & Malik C P, Phytochrome control of some oxido-reductases in germinating *Pinus roxburghii* pollen, *Pl Cell Physiol*, **20** (1979) 675-78.
10. Dhawan A K & Malik C P, Cyclic A M P control of some oxido-reductases during pine pollen germination and tube growth, *Phytochemistry*, **18** (1979) 2015-17.
11. Bhandal I S & Malik C P, Total and polar lipids biosynthesis during *Crotalaria juncea* Linn. pollen tube growth. Effect of gibberellic acid, indoleacetic acid and (2-chloroethyl)-phosphonic acid, *J exp Bot*, **31** (1980) 931-35.
12. Singh M B, Bhalla P L & Malik C P, Activity of some hydrolytic enzymes in autolysis of the embryo suspensor in *Tropaeolum majus*, *Ann Bot*, **45** (1980) 523-27.
13. Dhaliwal A S, Malik C P & Singh M B, Overcoming incompatibility in *Brassica campestris* L by carbon dioxide and dark fixation of the gas by self- and cross-pollinated pistils, *Ann Bot*, **48** (1981) 227-33.
14. Malik C P, Self-recognition and control of the incompatibility responses of *Brassica campestris* pollen, *Acta bot Indica*, **9** (1981) 1-13.
15. Sharma S, Singh M B & Malik C P, Relation of glycosidases to *Amaryllis vittata* pollen tube growth, *Pl Cell Physiol*, **22** (1981) 927-31.
16. Bhandal I S & Malik C P, Total and polar lipid biosynthesis during growth of *Crotalaria juncea* pollen tubes, *Phytochemistry*, **20** (1981) 429-32.
17. Bhalla P L, Singh M B & Malik C P, Studies on the comparative biosynthetic activities of embryo and suspensor in *Tropaeolum majus* L, *Z Pflanzen Physiol*, **103** (1981) 115-19.
18. Basra A S & Malik C P, Dark metabolism of CO<sub>2</sub> during fibre elongation of two cottons differing in fibre lengths, *J exp Bot*, **34** (1983) 1-9.
19. Malik C P, Regulation of developmental physiology of plant reproductive structures by carbon dioxide, *Phytomorphology*, **33** (1983) 122-28.
20. Basra A S & Malik C P, Development of the cotton fibre, *Int Rev Cytol*, **89** (1984) 65-113.
21. Malik C P, Metabolic control of pollen germination, in *Recent Advances in Pollen Research*, edited by J M Verghese (Allied Publishers, New Delhi) 1985, 25-44.
22. Basra A S & Malik C P, Non-photosynthetic fixation of carbon dioxide and possible biological roles in higher plants, *Biol Rev*, **60** (1985) 357-401.

## P N Mehra

### Hepaticae

The most significant work of Mehra is the postulation of "Condensation Theory" on the evolution of Marchantiales from the foliose ancestors passing through a Petalophyllum-like stage. The theory takes into account the structure of thallus as understood through making of transparencies, evidences from cytology, embryogeny and from fossil records. Evolutionary trends in the Hepaticae have been elucidated starting from Takakiales and Calobryales through foliose Jungermanniales culminating in the Marchantiales, in adaptation to 'xerophytic' conditions of life from hygrophilous ancestors. This theory is now accepted universally, replacing the older theories of progression or regression which seem untenable. Based on a number of premises, including the spore structure, the phyletic evolution of the Hepaticae has been traced, which is another significant contribution.

The cytology of a number of Indian Hepatics belonging to Calobryales, Jungermanniales, Marchantiales, and the Anthocerotae has been worked out. These groups stick to their respective basic chromosome numbers and show polyploids in very rare cases. A significant feature is the occurrence of an 'm'

chromosome in almost all the groups, with rare exceptions. Sex chromosomes have been discerned in a number of dioecious species like *Petophyllum indicum*, *Cyathodium flabellatum* and *Riccia frostii* in all of which they are distinctly heteromorphic, and in *Cyathodium tuberosum* in which they are sub-heteromorphic. In some other dioecious species like *Sewardiella tuberifera*, *Calycularia crispula*, *Stephensoniella brevipedunculata* and *Marchantia palmata*, the sex chromosomes are indistinguishable.

### Pteridophytes

Our work on the cytology and cytotaxonomy of the Himalayan ferns has been most comprehensive. Out of nearly 500 taxa distributed in this region, more than 375 have been investigated, most of them for the first time, which works out to about three fourths of the entire Himalayan fern flora. Numerous species complexes have been discovered and a large number of obligate apomicts whose cytological analyses were done on the bases of the pairing phenomenon of the 16-celled sporangia have been accomplished. The impact of cytological investigations, coupled with morphological features and anatomical characters, has led to the postulation of a system of phyletic

---

Emeritus Professor & Former Head, Department of Botany, Panjab University, Chandigarh 160014 and Research Adviser, Himachal Pradesh Agricultural University, Mashobra, Simla; Residence : Bunglow No 1055, Sector 27-B, Chandigarh-160027.



classification of the ferns as a whole, which were elaborated in my presidential address delivered at the Botany Section of the Indian Science Congress Session in 1961. A list of chromosome numbers of the Himalayan ferns indicating the apomictic taxa and their level of ploidy has been published.

Biosystematics of the *Adiantum lunulatum* complex occurring in the Eastern and Western Himalayas has been studied. In this species, as early as 1944, the mechanism for carrying forward the same chromosome number of this triploid apomict with ' $n$ ' = 90 in the gametophytic and sporophytic generation by the breakdown of premeiotic mitosis resulting in restitution of nuclei in the 8-celled sporangia which then show autosyndetic pairing at meiosis was elucidated for the first time in ferns. This has been subsequently found by later workers to be the most common phenomenon in apomictic ferns.

Stomatal patterns of 358 species belonging to 117 genera of the pteridophytes were investigated. From this evolutionary approach it has been concluded that out of the 13 different types, the "Psilophytaceous type" is the most primitive and all the others are derived on a plurality of lines. In ferns, 10 different patterns are observable.

### Gymnosperms

The work in gymnosperms centres around the genus *Ephedra* and the conifers. The salient features brought out in the former are: (1) the discovery of hermaphrodite 'flowers' in *E. intermedia* and their implication in the relationship of Ephedrales with other members of Gnetophytes, (2) establishment in 1946 of

a consistent basic karyotype in the genus based on investigations on seven species belonging to the section *Pseudobaccatae* (since confirmed by other investigators in other members of the genus). (3) determination of the basis of equality or inequality in the size of the male gametes, (4) discovery of a sizeable percentage of diploid pollen grains in the species investigated and their mode of formation as well as their implications in the origin of polyploids in the genus, and (5) discovery of the sex determining mechanism in *E. foliata* and allied species. A new efficient and easy method of *in vitro* germination of pollen grains on the natural mucilage secretion oozing out of the micropylar end of ovules of any species of the genus was developed and the cytotoxic effect of the drugs colchicine and sulfanilamide, commonly used in human ailments, on the formation of abnormal male gametes, was elucidated.

The highlight of our work on conifers is a detailed study of the karyology of conifers of India and some introduced exotics, which has been acclaimed widely. This work was presented at the fifth world Forestry Congress held at Seattle, USA, in 1961. The most significant deduction is the postulation of "Saturation Hypothesis" accounting for the general lack of polyploidy in the evolution of conifers, which has since been accepted by the contemporary workers. Another important contribution is in the resolving of *Abies* and *Juniperus* complexes in the entire range of the Himalayas which was in a state of confusion in the past. These complexes have been worked out in detail, morphologically, cytologically and anatomically. An illustrated monograph on these complexes occurring in the E. Himalayas has been published.

Unequivocal occurrence of hybrid swarms on a vast scale between the low-level fir (*Abies pindrow*) and high-level fir (*A. spectabilis*) in W. Himalayas has been established.

## Cytological Investigations

### (A) Monocotyledons

Alismataceae (the most primitive family in the group) shows the basic chromosome number  $x=6$  with median sub-median chromosomes. There is evidence of increase in number through fission at the centromere region in some species of *Alisma* and the allied genus *Sagittaria* giving rise to aneuploids with correspondingly increased number of near telocentric chromosomes.

Exceptionally large chromosomes are observed in *Trillium*, *Fritillaria* and *Paris*, and fairly large chromosomes in *Lilium*, *Polygonatum*, *Disporum* and *Allium*. The karyotypes analysed from their root tips show quite a few chromosome pairs which are heteromorphic. Heteromorphism seems to be associated with the large size of chromosomes, the alterations being due to their congestion in occupying relatively limited space within the nucleus. These plants show vegetative reproduction extensively through corms, bulbs and tubers involving dynamic alteration of chromosomes in number and structure in polymorphic populations adapted to different habitats and geographic areas. Special mention in this connection may be made of *Polygonatum*, *Disporum* and *Trillium* species.

A comprehensive illustrated monograph on the "*Orchids of Khasi and Jaintia Hills*" covering 60 genera and 158 species has been published. The broad conclusions from these studies are: The original basic

chromosome number of the family probably was  $x=5$ ; Cypripedieae with long chromosomes is the most primitive tribe; gradual reduction in the size of chromosomes has taken place during evolution; the most stabilized constitution of the modern orchids is at the octoploid level with  $n=19,20,21$ ; secondary association of bivalents is frequent; wobbling of 1 or 2 chromosomes occurs during speciation without impairing reproductive efficiency; accessory chromosomes are not uncommon; and sparse polyploid evolution is exhibited on present-day base numbers. Pollen specialization has occurred independently in various tribes from individual pollen grains—loose tetrads—tetrads—loose massulae—massulae—pollinia.

Cytological investigations were carried out on grasses of the Panjab plains, Western, Central and Eastern Himalayas, Assam and adjoining areas which cover nearly 600 species. A large number of genera and species have been worked out for the first time and numerous polyploid and aneuploid races, unknown before, have been discovered.

The tribes Andropogoneae ( $x=5$ ), Maydeae ( $x=5$ ), Panieae ( $x=9$ ), Arundinoid-Eragrostoid ( $x=10$ ), Festucoid ( $x=7$ ) and Bambusoid grasses ( $x=6$ ) have consistent basic numbers (indicated in parentheses). The size of chromosomes of Festucoids is the largest and that of Bambusoids, the smallest in the family. Polyploidy is rampant, to the extent of 70% or more. A number of species complexes have been discovered, of which the more interesting ones are *Saccharum spontaneum* complex  $n=20,21,22,27,28,29,30,32,33,34,36,37,40$ ; *S. bengalense* complex  $n=10,20,30,40$ ;



*Arundinella khaseana* complex  $n = 9, 27, 30, 36, 40$ ; and *A. nepalensis* complex  $n = 10, 20, 25, 27$ . The occurrence of B-chromosomes is quite frequent. Secondary associations of bivalents have been observed in many cases. Desynapsis, asynapsis, interlocking of bivalents and multivalent formations are noticed. An unusual feature observed is the occurrence of a complete or incomplete chromatin ring at diakinesis and even at later stages of meiosis in *Anthoxanthum odoratum* ( $2n = 20$ ). In a number of species, meiosis is irregular and apomixis is suspected.

The underlying basis of speciation within the family is alluded to allogamy, perennial habit, gregarious growth promoting hybridization, pre-disposition to polyploidy (i.e. easy adjustment of multiple genome to cytoplasmic-chromosomal relationships), and in certain cases apomixis.

Based on cytology and morphology and supplemented with anatomical data, especially of the leaf, a scheme for the phylogenetic evolution of the Gramineae was presented depicting evolutionary relationships between the various tribes. This was appreciated by Drs Hubbard and Bor—the two world authorities on grasses, who have for long been involved in this type of study.

#### (B) Dicotyledons

Our work on the cytology of tropical hardwoods covering the entire range of the Himalayas is regarded as the most comprehensive in the world. Nearly 650 species of woody elements which include 528 tree species have been worked out, mostly from meiotic preparations. Numerous papers have been published in international journals, culminating in a

monograph entitled "Cytology of Himalayan Hardwoods". Four hundred species and 66 genera have been investigated for the first time, which is a record; this aspect of the work was sadly neglected in the past. The highlights of these investigations are as follows.

Chromosome size in hardwoods is generally small, in fact much smaller than those of the conifers or the softwoods, in particular the Pinaceae. However, relatively larger chromosomes, though standing no comparison with pines, are met in the Magnoliaceae and a few other genera distributed sporadically in various families. The small chromosome size is compensated by their large numbers, thus balancing the chiasma frequency and hence variability index.

The lowest base number  $x = 7$  is still present in a small number of genera like *Shorea*, *Helicia*, *Cassia*, *Dobinea* and a few others, whereas majority of the genera possess high base number like *Bischofia*  $x = 98$ ,  $x = 49$ , *Tilia*  $x = 41$ , *Salmaal*  $x = 40$  and so on, although the mode is at  $x = 11, 12$  and  $13$  in a majority of the taxa. Obviously, the high base numbers are a result of compounding of chromosomes or their palaeoploid origin. Compounding of chromosomes is evident in Magnoliaceae with uniformly  $x = 19$ , which has been interpreted as a synthesis of  $x = 7$  and  $x = 12$  met in closely allied families. Likewise, Pomoideae (Rosaceae) possess uniformly  $x = 17$ , which may be the result of synthesis of  $x = 9$  and  $x = 8$  occurring in the two other tribes of the family, namely, Prunoideae and Spireoideae. An interesting feature is that certain genera and even whole families stick to the same basic chromosome number like members of the Magnoliaceae  $x = 19$ , Aceraceae

$x = 13$ , Rubiaceae  $x = 11$ , Lauraceae and Fagaceae  $x = 12$ , etc., compared to other genera, which are polybasic; the characteristic ones are Rhamnus  $x = 10, 11, 12, 13$ , zyzophus  $x = 10, 12, 13$ , Pistacia  $x = 12, 14, 15$ , etc.

The fallacy of regarding polyploidy to be rare in tree elements has been rectified on the basis of our investigations. This is of the order of 24.6% in contrast to 33% in Angiosperms in general. Polyploid evolution has been both interspecific and intraspecific. A hypothesis designated as "The Saturation Hypothesis" has been postulated which visualizes that in certain taxa, the optimum physiological balance for proper functioning in regard to growth and vitality has been reached at the diploid level, while in others, because of the capacity of production of diversified genomes, polyploid evolution could occur with advantage. The earlier fallacy of relating polyploid evolution in whole floras to climate with disregard for the intrinsic capacity of the constituent elements for such polyploid evolution is pointed out. It would be correct to draw conclusions only while considering the distribution of specific genera in relation to the climate.

## Morphogenesis

### Ferns

Morphogenetic studies *in vitro* have been carried out on 10 species of ferns (6 diploid, 4 tetraploid and 1 hexaploid). It has been possible to break the barriers between the gametophytic and sporophytic generations through experimental manipulation of the culture medium.

It has been possible to bypass sexual fusion to form sporophytes (or their parts) and alternatively meiosis to form

gametophytes. Every cell of the sporophyte or gametophyte is totipotent, irrespective of the number of genomes contained within it and can be stimulated as such or through their calli to form either of the two generations, or in the case of gametophytes only roots or only leaves.

On the basis of these studies, 'Gene Block Hypothesis' in differentiation has been postulated which seems to be equally applicable to Hepatics and Mosses. Also, "Genetic Theory of Alternation of Generations" is postulated and it has been reasoned on the basis of the present experimental studies and the morphocytological situation in Algae that neither the Homologous Theory nor the Antithetic Theory of Alternation of Generations correctly interprets the situation as it exists. The "Genetic Theory of Alternation of Generations" is the most plausible explanation.

### Fruit Trees

Complete success has been achieved in almond (differentiation of plants through callus cultures), apple and pear (differentiation through callus and embryogenesis), pomegranate (differentiation through callus, embryogenesis and direct regeneration from explants) and chikoo (differentiation through callus and regeneration). Apart from the success in clonal propagation of the above-mentioned fruit trees in general, the highlights of academic interests are: (1) differentiation of callus to form either isolated roots or leaves or shoots to the exclusion of all the other organs, which fact amply supports the 'Gene Block Hypothesis' postulated earlier by us in Fern System, (2) the cells embarking on embryogenesis become isolated from the general callus mass, (3) embryoid development not necessarily



through the *in vivo* pathway, (4) totipotency of every organ, including the petals (in pear) or the fruit tissue (apple and pear), and (5) bizarre cell types in calli (almond, pear) obviously influenced by the nature of the medium. Another point worth mentioning is that the chromosome numbers in cells of these materials, i.e. almond ( $2n = 16$ ), apple and pear ( $2n = 34$ ), pomegranate ( $2n = 16$ ), and chikoo ( $2n = 26$ ) in prolonged callus cultures tend to become polyploids or aneuploids, rendering them unsuitable for successful organogenesis or embryogenesis.

### Forest Trees

#### Softwoods

The species tackled are *Pinus gerardiana* (the chilghoza pine), *P. roxburghii* (the chir pine), *Cedrus deodara* (the Himalayan deodar) and *Picea smithiana* (W. Himalayan spruce). In addition, investigations were also conducted on *Cryptomeria japonica*—an exotic (native of China and Japan), which does very well in the E. Himalayan Mountains around Darjeeling.

In *Picea smithiana*, multiple shoot-buds were differentiated on each cotyledon of young embryo on MS + 4 mg/litre kinetin. In *Cedrus deodara*, such adventive shoot-buds, as well as needles, were differentiated on hypocotyl as well cotyledons on MS + 3 mg/litre kinetin. Another interesting feature noticed in the calli of *Cedrus deodara* was the differentiation of peculiar multicellular and branched 'tracheidal hairs' with bordered pits and reticulate thickenings from the surface of compact calli raised on BMS + NAA + K.

#### Hardwoods

Our work on poplars is highly interesting and productive. *Populus ciliata* and *P. alba* are both natives of the Himalayas. The former because of its soft wood is used commercially in the matchwood industry. Further, because of its quick growth, it is an ideal material for re-afforestation programmes. It is comparable in every way to the triploid aspen (*P. tremula*) used extensively in the advanced countries for timber purposes.

Methods have been evolved for prolific multiplication of plants from elite tree(s) using *in vitro* technique. Leaf-discs from young leaves and calli obtained from vegetative parts of 40-year old trees (stem, leaf, apical bud and axillary buds) have been induced to give a bumper crop of young plants, even in one season, by the application of growth hormones BAP and IBA in suitable proportions. Even the growing apical buds can be made to undergo prolific sprouting through the application of appropriate quantity of BAP. These can be excised and rooted to form full-fledged plantlets.

This is perhaps our most important contribution towards achieving clonal multiplication of elite trees on a vast scale in the hardwoods.

### Selected Publications

1. Mehra P N, Cytological investigations of apogamy in *Adiantum lunulatum*, *Proc natn Acad Sci India*, **14** (1944) 189-204.
2. Mehra P N, A study of karyotypes and occurrence of diploid male gametophytes in some species of the genus *Ephedra*, *Proc natn Acad Sci India*, **16** (1946) 259-86.
3. Mehra P N, Occurrence of hermaphrodite flowers and development of female gametophyte in *Ephedra intermedia*, *Ann Bot (N S)*, **14** (1950) 165-80.

4. Mehra P N & Vashist B R, Embryology of *Petalophyllum indicum* Kash. and a new suggestion on the evolution of a thalloid habit from foliose forms, *Bryologist*, **58** (1950) 89-114.
5. Mehra P N, Colchicine effect and the production of polyploid spermatozoids in the prothalli of *Dryopteris subpubescent* and *Goniopteris prolifer*, *Ann Bot (N S)*, **16** (1952) 49-56.
6. Mehra P N & Khoshoo T N, Cytology of conifers. I, II, *J Genet*, **54** (1956) 165-80, 181-85.
7. Mehra P N, A new suggestion of the origin of thallus in the Marchantiales. I. The thallus structure. II. The theory, *Ann J Bot*, **44** (1957) 505-13, 573-81.
8. Mehra P N & Mittal T C, Indian substitutes of male fern in pharmacognosy, *Nature, Lond*, **186** (1960) 722-23.
9. Mehra P N, Cytology and breeding of conifers, *Invitation Pap. 5th World Forestry Cong (USA)* **2** (1961) 728-33.
10. Mehra P N, Cytological evolution of ferns with particular reference to Himalayan forms, *Presidential Address, Botany Section, 48th Session, Indian Science Congress, Roorkee*, 1961, 1-24.
11. Mehra P N, Phyletic evolution in Hepaticae, *Phytomorphology (Panchanan Maheshwari Memorial Vol)*, **17** (1967) 47-58.
12. Mehra P N, Palynology of Archegoniatae—An evolutionary approach. First Annual Lecture, Palynological Society India, *J Palynol*, **4**(10) (1968) 56-72.
13. Mehra P N Conquest of land and evolutionary patterns in early land plants, *15th Sir Albert Charles Seward Memorial Lecture, Birbal Institute, Indian J Genet Pl Br*, **26**(2) (1968) 97-111.
14. Mehra P N, Cytogenetical evolution of conifers, *2nd Mendel Memorial Lecture, Indian J Genet Pl Br*, **28**(2) (1968) 97-111.
15. Mehra P N, Evolutionary trends in hepaticae, *Maheshwari Memorial Lecture, Phytomorphology*, **19** (1969) 203-18.
16. Mehra P N, Cytogenetical evolution of hardwoods. Presidential Address 1st All India Congress Cytology and Genetics, *Nucleus*, **15** (1972) 64-83.
17. Mehra Asha & Mehra P N Organogenesis and plantlet formation *in vitro* in almond, *Bot Gaz*, **135** (1974) 61-73.
18. Mehra P N, Some aspects of differentiation in Cryptogams, *3rd P R White Memorial Lecture, Res Bull Panjab Univ (NS)*, **23** (1975) 221-42.
19. Mehra P N & Jain K K, *Abies and juniperus complexes in the E. Himalayas with observations on Larix griffithii Hook. f. and Tsuga dumosa Eichler* (Saraswati Press, Calcutta) 1976, pp 143.
20. Mehra P N, *Cytology of Himalayan hardwoods* (Saraswati Press, Calcutta) 1976, pp 248.
21. Mehra P N, *Cytology of East Indian Grasses* (Rajbandhu Industrial Co., Delhi) 1982, pp 248.
22. Mehra P N, *Cytology of orchids of Khasi and Jaintia Hills* (Rajbandhu Industrial Co., Delhi) 1983, pp 118.
23. Mehra P N, Evolution of the spore through the ages. *Foundation Lecture, First Indian Palynological Congress*, 1974.
24. Mehra P N & Soni P L, Stomatal patterns in Pteridophytes—An evolutionary approach, *Proc Indian natn Sci Acad*, **B49**(2) (1983) 155-203.



## R Misra

The present account presents an overview of my investigations conducted during the last 50 years. The selected publications listed at the end provide an idea of the species, communities and ecosystems examined and experimented upon. The generalizations made are, therefore, based on conceptual analysis generated by the entire work.

My first series of observations supported by culture experiments started in and around lake Windermere in UK<sup>1</sup>. The dependence of rooted aquatic plants on nutrients largely absorbed from the bottom mud was established beyond doubt. Both low and high organic contents of the silt deposit led to its sterility. The rate of decomposition of the organic debris and the silt fertility are reflected by the flux of redox-potential. Form variations so common in the plants are due to difference in light intensity obtained at different depths and these are mostly phenotypic (ecophenes), though some of the species generate ecotypes. These observations as extended to Indian situations revealed habitat adaptations of plants with varying genotypic and phenotypic plasticity in response to light, photoperiod, moisture, nutrient, temperature regime, phases of growth and development, etc., in the case of aquatic, marsh and land plants. Many of the processes and patterns of distribution were explained on the basis of interactions

of the phenomena through time and space<sup>2-5</sup>.

Community development through plant succession involves spiralling alterations in the habitat and plant adaptations, distribution and adjustments with systemic efficiency of lowering entropy and maximum diversity. The input and output fluxes stabilize organic productivity, reflecting economy of the bio-geochemical flows<sup>6-10</sup>. The seasonal pattern of plant communities is interpreted afresh within the scheme of the long-term phenomenon of succession<sup>6</sup>.

Whether we look to ecological phenomena as isolated series of single events or in their aggregates or through the holistic concept of ecosystem, in all such cases interactions of forms, factors and functions are amenable to modelling of biological or organic productivity<sup>3</sup>. Our participation in the International Biological Programme (1964-1974) led to a series of investigations of organic productivity in grassland, shrubland, forest and aquatic ecosystems stressed by human activities of different degrees. New hypotheses and interpretations were given to food chains, trophic structure on land and in water and flow of energy and nutrients<sup>7-10</sup> within the Indian ecosystems.

My participation in the initiation of the UNESCO Man and Biosphere Programme

---

Formerly, Professor & Head, Department of Botany, Banaras Hindu University; Residence : DII/4, Indian Agricultural Research Institute, New Delhi-110012.

and its launching in 1972 focussed our attention on the stresses on the Indian ecosystems caused by human activities<sup>11,12</sup>. High entropy technology destabilizes natural ecosystems, so that they lose biological productivity and resilience and become fragile. A case study of the processes going on in the degradation of the Vindhyan series of ecosystems<sup>13</sup> was presented at the Nairobi UNEP symposium on desertification and the impact of urbanization and industrialization on the natural vegetation of the region was discussed at the 1981 Indian Science Congress. These studies highlighted the need for establishing biosphere reserves in India within the global chain of such reserves for investigations and scientific management of stressed ecosystems all over the world as pursued by the IUCN<sup>9,13</sup>.

A logical follow up of these investigations led to the discovery that natural or highly stressed ecosystems holding different amounts of standing biomass, such as grass, shrub, forests or low input agriculture yield about 15 tonnes of (dry) organic matter per year per hectare in the Vindhyan region<sup>11,12</sup>. However, the stability of the systems depends on accumulation of organic matter over the years as the capital base. Systems overstressed by heavy grazing, high input and hence energywise less efficient agriculture, and heavily exploited forests, are low yield and fragile systems.

Sustainable human population has to be lowered day by day with our changing high entropy technological life-style as the ecosystems become fragile and more and more open for input and output flows of energy and materials from the adjacent ones. Ecology as a tool for managing ecosystems cannot cope with the situation

unless non-material dimensions of human behaviour are internalized within the ecosystem<sup>5,15</sup>. Such a system will not be amenable to mathematical modelling because of non-material attributes of life.

The situation calls for amending our life-style from high consumption to low consumption. We have to reorient science and technology in order to process the organic productivity of the biosphere, which is far in excess of the food requirement of the world population<sup>13,14</sup>. Biotechnology must not be narrowed down to genetic engineering.

The managers of resource use should have sound knowledge of ecology<sup>4</sup>. There is no discipline of knowledge which is not useful for appreciating the complexity of the environment. But we need ecologists to work out the dynamics of linkages within the network interactions of human activities. All the activities are not quantifiable. Social, economic, political, spiritual, aesthetic and moral values must be fused with those of science and philosophy in order to save the world from the nuclear holocaust and much worse from ecological scarcity situations. Thinking globally and acting locally—a slogan given by UNEP—IUCN calls for a biospheric world order based on conservation. Gandhi's life-style as a model for environmental conservation is being increasingly appreciated the world over<sup>3,15</sup>. An army of expert ecologists has to be raised and mass education has to be pressed through all possible media for reorientation of our value system. A post-graduate comprehensive environmental science course has to be outlined for developing leadership in this area<sup>15</sup>.



### Selected Publications

1. Misra R, Edaphic factors in the distribution of aquatic plants in the English Lakes, *J Ecol*, **36** (1938) 411-45.
2. Misra R, Environment, adaptation and plant distribution, *Proc Indian Sci Cong 46th Part K K (Presidential address)*, 1959, 96-107.
3. Misra R, Form, function and factors in ecology (Birbal Sahni Medal award lecture), *J Indian bot Soc*, **46** (1967) 144-53.
4. Misra R & Singh K P, Ecology in India—A bird's eyeview, *Indian bot Soc Golden Jubilee Vol*, **50A** (1970) 589-601.
5. Misra R, My lessons of environmental education, in *Education for environmental planning and conservation*, edited by D Bandhu and N L Ramanathan (Indian Environmental Society, New Delhi), 1981, 49-56.
6. Misra R, The status of plant communities in the upper Gangetic plain (Presidential Address), *J Indian bot Soc*, **38** (1959) 1-6.
7. Misra R, Singh J S & Singh K P, A new hypothesis to account for opposite trophic biomass structure on land and in water, *Curr Sci*, **37** (1968) 381-82.
8. Misra R, Energy transfer along terrestrial food chains, *IBP News, Lond*, No. 12 (1969) 20-22.
9. Misra R, Primary production of Chakia forest and IBP/PT study of organic productivity and nutrient cycling in monsoon forests, grasslands and croplands, *IUCN Publications, New Series No 17* (1970) 230-39.
10. Misra R, A comparative study of net primary productivity of dry deciduous forest and grassland of Varanasi, in *Tropical ecology emphasizing organic production*, edited and published by F B Golley, Athens, USA, 1971, 279-93.
11. Misra R, Soil fertility and nutrient dynamics in forest, grassland and cropland ecosystems and impact of industry on the soil, in *Proceedings, Symposium on Soil Productivity*, edited by N R Dhar (National Academy of Sciences, India), 1972, 269-76.
12. Misra R, Indian Savannas, Chapter 7 in *Tropical savannas*, Vol 13, *Ecosystems of the world*, edited by F Bourlier (Elsevier Scientific Publishing Co, Amsterdam) 1983.
13. Misra R, Environmental aspects of land use in semiarid regions of Asia, in *Environment and development*, edited by M Dwarkin (SCOPE Indiana, USA) 1974, 32-42.
14. Misra R, Effect of urbanisation and industrialization on the natural vegetation in India, in *Impact of the development of science and technology on environment*, edited by A K Sharma and A Sharma (Indian Science Congress Association, Calcutta) 1981, 62-77.
15. Misra R, University education in environmental science, *INSA Golden Jubilee—Sadasivan Endowment Lecture, Varanasi, Proc Indian natn Sci Acad*, **B** (1985) in press.

## H Y Mohan Ram

Wonderment about plants was aroused in me during my undergraduate days by my teachers Dr M A Rau and Mr B N N Rao, who took students for botanizing in the picturesque town of Mysore. From curiosity to disciplined action became possible under the tutelage of two great teachers—P Maheshwari at Delhi and F C Steward at Cornell.

Writing about one's own work is not easy. But I am glad to look back and take stock of what has emerged from my laboratory. My early efforts were devoted to the study of seed development in the Acanthaceae and reproductive biology of banana. What follows is a gist of the main findings in certain areas of botany made by me and by 25 research students who have worked with me. I am happy to be a teacher and I deeply acknowledge the contributions made by the research scholars in these studies.

### Modification of Sex Expression

Chemical manipulation of sex expression has been investigated by our group using *Cannabis sativa* (dioecious) and *Ricinus communis* (monoecious). In *Cannabis sativa*, sex becomes distinguishable only when flowers appear. The male and female flowers are morphologically quite different. A thorough cytological study has indicated

the absence of a heteromorphic pair of sex chromosomes. Male flowers (functionally and morphologically identical to natural ones) can be induced in the female *Cannabis* plants by the application of gibberellins (GA's) and antiethylene agents such as silver nitrate ( $\text{AgNO}_3$ ), silver thiosulphate anionic complex (STS), amino ethoxyvinyl glycine (AVG) and cobalt chloride ( $\text{CoCl}_2$ ). Fertile female flowers can be induced in male plants by ethephon (2-chloroethane phosphonic acid) and NIA 10637 (ethyl hydrogen-1-propylphosphonate). We have hypothesized that in *Cannabis*, GA and ethylene act as male and female hormone respectively and that the expression of sex is controlled by a balance between their endogenous levels. ABA is able to overcome the GA-induced male flower formation.

It was of interest to identify biochemical markers for sex in the vegetative state. A detailed survey demonstrated that there are no qualitative differences in the soluble amino acids or in the isoperoxidases or isoesterases in the vegetative shoot tips of male and female *Cannabis* plants. The female parts have a much higher specific activity of peroxidases, whereas the male parts have a substantially higher specific activity of amylases. In the flowering male shoot tips and in isolated male flowers, three entirely new ("male specific")



isoperoxidases (in addition to those that are common to both male and female flowers) were noted. These also appeared in the induced male flowers on female plants.

In the female-240 line of *Ricinus communis*, the primary terminal inflorescence bears only female flowers. Injection of  $GA_3$ ,  $AgNO_3$  or  $CoCl_2$  to the hollow internodes can induce male flowers, enabling selfing and maintenance of this female line.

The molecular mechanism of the role of hormones in sex expression is yet to be elucidated.

The application of the technique of chemical modification of sex expression has the potential to produce seeds that will bear only female plants in the economically important dioecious plants.

### Flower Physiology

Some basic studies have been carried out using excised inflorescences of lupine, gladiolus, chrysanthemum, marigold, lantana and flowers of rose. This work has not only provided methods of prolongation of vase-life but has also given an insight into the mechanisms of flower opening, petal growth, petal colour and senescence.

#### Mechanism of flower bud opening in gladiolus

In gladiolus, the system of overlapping outer bracts (which completely enclose the flower buds) and their gradual separation represents a system programmed for sequential exposure of successive buds to light. The outer bract acts as a natural qualitative light filter and regulates the production of  $\alpha$ -amylase and petal growth by a red/far-red control.  $\alpha$ -Amylase is formed exclusively in the petal epidermis. On perception of light, the enzyme is

transported to the ground parenchyma where it hydrolyses the extensive starch reserves. Interestingly, the epidermal cells become competent to be induced by light only when they attain a stage of differentiation, manifested by the bulging of the outer radial wall to form a microlens, which focuses light specifically on to the nucleus. Sugars released through  $\alpha$ -amylase activity are involved in petal growth through the development of an osmotic and sink potential. A critical stage in flower bud growth in the spike of gladiolus which is initiated by gibberellic acid and sustained by sucrose has also been identified. This corresponds to the stage at which separation of the outer bract occurs. In gladiolus, evidence has been obtained to demonstrate that stamens play a role in petal expansion, presumably through the production of gibberellins.

#### Expansion of ray florets in chrysanthemum

An elegant method (consisting of floating of ray florets in test solution in petri plates) has been developed to study the expansion of ray florets of chrysanthemum.  $KCl$  +  $GA_3$  + sucrose cause up to 82% increase in elongation. It is inferred that the increased turgor resulting from sucrose-promoted potassium uptake along with  $GA_3$ -caused tissue extensibility accounts for the enhanced floret growth.  $N$ ,  $N'$ -dicyclohexyl carbodiimide (DCCD, a potent inhibitor of membrane-bound ATPase) and CCC (an inhibitor of gibberellin biosynthesis) inhibit ray floret growth.

#### Significance of flower colour changes in Lantana

In a colour variant of *Lantana camara* selected for study, pink buds, yellow newly opened flowers and ageing orange, scarlet and magenta flowers are found in the same

inflorescence. The flower pigments are identified as delphinidin monoglucoside and  $\beta$ -carotene. Thrips are regular pollinators and are attracted to only yellow flowers. Pollination triggers rapid anthocyanin synthesis. The post-pollination shift in petal colouration is caused by the masking of carotenoids by differential amounts of anthocyanin. Thus, chromatic changes play a role in conserving pollinator energy.

#### **Pre- and post-storage treatment of harvested spikes of gladiolus**

Spikes of gladiolus are generally harvested at the tight bud stage, but these are damaged considerably during transport. However, if the spikes are harvested at the green bud stage (one week earlier than the tight bud stage) only 50% of the buds open out. A pre-storage treatment of green bud spikes with sucrose ensures good opening. As a post-storage treatment, both sucrose and  $GA_3$  are needed. A pulse treatment with sucrose +  $GA_3$  is superior to continuous treatment for promoting opening of buds in cold-storage spikes.

#### **Role of ethylene and antiethylene compounds in flower senescence**

It is now well-established that ethylene triggers flower senescence. We have shown that antiethylene compounds such as  $CoCl_2$ ,  $NiCl_2$  and  $FeCl_2$  promote vase-life and increase the size of cut capitula in chrysanthemum and marigold. Addition of sucrose enhances the vase-life further. A useful finding is the retardation of senescence of fully open capitula of chrysanthemum by 10-15 days by the spray application of  $CoCl_2$  or  $NiCl_2$  to potted plants.

#### **Biology of Aquatic Plants: Knowledge Gained from Whole Plant Culture**

A large number of aquatic plants are recorded from India, but only a few have been studied in detail, probably because of inconvenience or inaccessibility. Our group took up the cultivation of aquatic plants in axenic cultures, as we recognized that this technique provides unlimited opportunities for studying growth, nutrition, flowering and reproduction.

Several long-standing questions in the biology of water plants have been answered by our studies. We have noted high rates of growth, extraordinary propensity for regeneration and vegetative propagation, obligate requirement for sucrose, non-essentiality of vitamins, necessity of chelating agents (EDTA, EDDHA) and production of an enormous genetic variability in the populations (including "bushy seedlings") under culture conditions.

The bladderworts (*Utricularia*) are endowed with special devices to trap insects. There have been claims that nitrogen derived from the insect prey is an obligate requirement for growth and flowering of these plants. Our work has shown that *Utricularia inflexa* var. *stellaris* can grow, flower and set seed even on a medium containing nitrate nitrogen. Flowering in this plant occurs under short days (20 cycles of 16 h dark 8 h light).

Heterophylly (formation of several kinds of leaves on the same individual) is a characteristic feature of many amphibious angiosperms. The classical *Limnophila indica* bears pinnately-dissected leaves, arranged in whorls under water and entire, ovate and opposite-decussately arranged leaves and flowers on the aerial shoots.



Absciscic acid (ABA,  $10^{-7}$  and  $10^{-6}M$ ) induced typical aerial leaves and precocious flowers even in the submerged shoots. The effects caused by ABA may be interpreted as responses to induced stress.

While studying the effect of cytokinins on the development of *Ceratophyllum*, we noted that the degree of dichotomy and the length of leaf segments were directly related to the concentration of benzyladenine. The fact that the delimitation of the species within this genus is based on the degree of leaf dichotomy suggests that the different species must be producing varied amounts of endogenous cytokinins.

*Neptunia oleracea* is a tropical aquatic legume that produces a large biomass and fixes a high quantity of nitrogen. Recognizing its potential as a biofertilizer in the paddy fields, an attempt has been made recently to establish regenerants from excised root, hypocotyl and cotyledons with the objective of selecting useful variants. Using benzylaminopurine ( $10^{-6}M$ ), it has been possible to obtain a callus capable of differentiating shoot buds over 29 passages. Rooted plants have been transferred to pond conditions which have become established and have developed nodules.

The *in vitro* techniques developed by us for aquatic plants can be applied for the study of problems concerning heavy metal uptake, biomonitoring and conservation.

### **Tissue Culture of Legumes and Bamboo**

The use of tissue culture in genetic manipulation of legumes is severely handicapped by poor regeneration

response. We have taken up studies on developing continuously growing and differentiating tissue cultures from economically important legumes and have demonstrated a high incidence of whole plant regeneration in pigeon pea (*Cajanus cajan*), winged bean (*Psophocarpus tetragonolobus*), sunn hemp (*Crotalaria juncea*), *Sesbania sesban* and *Sesbania grandiflora*. It is envisaged that the methods developed will provide a means for obtaining regenerated plants for increasing selection of somaclonal variants for traits such as tolerance to drought and salinity and resistance to diseases and insects.

Promising results have accrued from the recent work on the tissue culture of bamboos. Multiple plantlets have been obtained from seeds and explanted tissues. This finding holds the possibility of producing large-scale planting material to meet the increasing demand for bamboos which are principal materials for construction and implements for the tribal people and raw materials for paper and cellulose.

### **Effect of Growth Regulators on Reproductive Development**

To exploit hybrid vigour and to overcome the laborious process of emasculation, hand pollination and bagging, scientists have been toying with the idea of using chemical compounds that can selectively induce male sterility without affecting seed fertility or seed quality. A detailed study carried out by our group using Mendok (sodium 2,3-dichloroisobutyrate), Dalapon (sodium 2,2-dichloropropionate) and morphactins on wheat and linseed indicated that a temporary period of developmental or functional male sterility can be obtained,

but none of the compounds is reliable as a specific male sterilising agent for commercial application.

The capitulum of Compositae bears a large number of florets in a specific pattern on its receptacle. In *Calendula officinalis*, secondary and often tertiary capitula are observed in nature. In our study to explain this phenomenon, we found that it was possible to transform a floret primordium, which had committed and limited developmental plan into a secondary inflorescence with a greater morphogenetic potentiality through the application of GA<sub>3</sub>. The application of morphactin (fluorene-9-carboxylic acid) to the young shoot tips suppressed floral development to various degrees, often resulting in the formation of totally barren capitula. These barren capitula can be excised and cultured to show the recrudescence of morphogenetic capacity. This work has been repeated with the demonstration of full grown plants from excised barren capitula of *Taquetes*.

### Other Current Studies

A major biological enigma in the bamboos is their long flowering cycles and death after gregarious flowering. Experiments are underway to induce sporadic flowering and test-tube flowering.

Of late, interest has been evinced in the production of potato from true seed to reduce the cost of cultivation, storage, transportation and to overcome virus diseases. Good seed production requires a thorough knowledge of reproductive biology. We have undertaken a monographic work on this aspect, which is nearing completion.

### Future of Botany

Botany is the science of survival. Plant world abounds in exciting diversity and offers challenging problems and opportunities for original research, which can be tackled with modern tools and techniques.

### Selected Publications

1. Steward F C & Mohan Ram H Y, Determining factors in cell growth: Some implications for morphogenesis in plants, *Adv Morphogenesis*, **1** (1961) 189-265.
2. Mohan Ram H Y, Ram Manasi & Steward F C, Growth and development of the banana plant. 3A. The origin of the inflorescence and the development of the flowers. B. The structure and development of the fruit, *Ann Bot (NB)*, **26** (1962) 657-73.
3. Mohan Ram H Y & Satsangi Asha, Induction of cell division in the mature endosperm of *Ricinus communis* during germination, *Curr Sci*, **32** (1963) 28-29.
4. Rustagi P N & Mohan Ram H Y, Evaluation of Mendok and Dalapon as male sterilising compounds in linseed, *New Phytologist*, **70** (1971) 117-30.
5. Doreswamy R & Mohan Ram H Y, Studies on growth and flowering in axenic cultures of insectivorous plants. II. Induction of flowering and development of flower in *Utricularia inflexa*, *Z Pflanzenphysiol*, **65** (1971) 315-25.
6. Mohan Ram H Y & Jaiswal V S, Induction of male flowers on the female plants of *Cannabis sativa* by gibberellins and its inhibition by abscisic acid, *Planta*, **105** (1972) 263-66.
7. Mohan Ram H Y & Jaiswal V S, The possible role of ethylene and gibberellins in flower sex expression of *Cannabis sativa* in *Proceedings of 8th International Conference on Plant Growth Substances* (Hirokawa Publishing Co., Tokyo) 1973, 987-96.
8. Mehta Usha & Mohan Ram H Y, Modification of the capitulum in *Calendula officinalis* by chlorflurenol, *Phytomorphology*, **28**(3) (1978) 351-69.
9. Sarath G & Mohan Ram H Y, Comparative effect of silver ion and gibberellic acid on the induction of male flowers on female *Canabis* plants, *Experientia*, **35** (1979) 333.



10. Rao I V Ramanuja & Mohan Ram H Y, Induction of amylase in the petal epidermis of gladiolus by light on separation of the enveloping bracts, *Proc Indian Acad Sci*, **89** (1980) 323-30.
11. Sett Rina & Mohan Ram H Y, Induction of male flowers in a pistillate line of *Ricinus communis* L. by silver and cobalt ions, *Planta*, **149** (1980) 413-15.
12. Mohan Ram H Y & Sett Rina, Induction of fertile male flowers in genetically female *Cannabis sativa* plants by silver nitrate and silver thiosulphate anionic complex, *Theor appl Genet*, **62** (1982) 369-75.
13. Pardha Saradhi P & Mohan Ram H Y, Correlated promotion of ray-floret growth in chrysanthemum by potassium chloride, gibberellic acid and sucrose, *Proc Indian Acad Sci*, **91** (1982) 101-6.
14. Mehta Usha, Rao I V R & Mohan Ram H Y, Somatic embryogenesis in bamboo, in *Proc V Int Cong Plant Tissue & Cell Culture*, Tokyo, Japan (1982) 109-10.
15. Mohan Ram H Y & Rao Sunanda, *In vitro* induction of aerial leaves and of precocious flowering in submerged shoots of *Limnophila indica* by abscisic acid, *Planta*, **155** (1982) 521-23.
16. Sriram N & Mohan Ram H Y, Sex associated differences in peroxidases and ethylene production and their modification by ethephon treatment in the flowers of *Cannabis sativa* L. *Curr Sci*, **53** (1984) 735-39.
17. Mohan Ram H Y & Ramanuja Rao I V, Physiology of flower bud growth and opening, *Proc Indian Acad Sci (Plant Sci)*, **93** (1984) 253-74.
18. Mohan Ram H Y & Mathur Gita, Flower colour change in *Lantana camara*, *J exp Bot*, **35** (160) (1984) 1652-62.
19. Hari Gopal B & Mohan Ram H Y, Systematic significance of mature embryo of bamboos, *Pl Syst Evol*, **148** (1985) 239-46.
20. Bala R, Rao I V R & Mohan Ram H Y, Influence of stamens, gibberellic acid and sucrose on corolla growth in gladiolus, *J Pl Physiol*, **122** (1986) 87-92.

## Y S Murty

### (I) Morphology, Nodal Anatomy, Floral Anatomy and Embryology

(1) *Piperales* : Twelve papers were published on vegetative morphology, anatomy, floral anatomy and embryology of 25 species belonging to five genera of Piperaceae and Saururaceae. From anatomical evidence, the placentation in *Peperomia* has been interpreted as sub-basal, which was accepted and quoted by Eames in his book 'Morphology of the Angiosperms' (1961). Further, the nature of the sterile stigma (beak), hypopeltate nature of the bract, bicarpellary condition of the ovary in *Peperomia*; adnation of stamen traces with carpellary dorsals in *Piper*; tricarpeal nature of the ovary, sub-basal position of the ovule in *Piper* and *Pothomorphe* marginal position of placentae and imperfectly closed condition of the carpels on ventral side in *Saururus*; parietal placentation and adnation of stamen carpellary bundles, in *Houttuynia* were reported.

Nodal anatomical studies helped in considering the opposite and whorled condition of leaves in *Peperomia* as derived and intrapetiolar membranous structure as representing axillary stipules in *Houttuynia*.

From embryological studies, the phylogenetic significance of the formation of evanescent walls observed during

megasporogenesis and longitudinal division of fertilized egg were reported in *Peperomia*. Embryo sac of *Piper quadrichaudianum* was interpreted as belonging to Fritillaria type and Polygonum type of embryo sac was confirmed in *Houttuynia*.

Separation of *Peperomia* from Piperaceae and constitution of Peperomiaceae were recommended.

(2) *Capparidaceae* : Study of the floral anatomy of normal and abnormal flowers of *Gynandropsis gunandra* provided evidence to discount the earlier interpretations about the number of carpels, bract, position of sepals, stamen supply.

Abnormalities like sepalody, petalody, staminody, pistillody and adnation of different floral parts were described.

(3) *Tamaricaceae* : From the floral anatomical study of seven species of *Tamarix* and one species of *Myricaria*, it was considered that obdiplostemony reported in these members and Troll's concept of paracarpy could not be supported. Unilacunar node was reported.

(4) *Ericaceae*—*Erica* : Studies on organography and floral anatomy of 54 species of *Erica*, mostly African, have revealed the presence of obdiplostemony, spur type of appendages on the stamens,



single traced sepals, petals and stamens. The book-like nature of stamen bundle has been interpreted as being due to inversion of the anther during development. Three traced sepals in some species, variable position and number of carpels, anther position and spur were utilized in discussing the inter-relationships of the species.

(5) *Acanthaceae*—*Barleria* : Pharmacognostic study of root, stem and leaf of three medicinally important species of *Barleria* was made to facilitate identification of crude drug samples.

(6) *Verbenaceae*—*Clerodendrum* : Floral anatomy of five species of *Clerodendrum* was studied and a vascular trace for the missing fifth stamen was reported in *C. inerme*. The placentation was considered axile in the base and parietal above. The conspicuous portion beyond the place of attachment of ovules, which takes almost half a round to cover the ovules, has been interpreted as the sterile part of the carpellary margins.

(7) *Apocynaceae*—*Lochnera pusilla* : The anatomy of the node, flower and embryology, including the seed coat structure have been described. The nectaries have been interpreted as carpallodes. Anatomically, the placentation is considered axile. Caecae-like endosperm haustoria have been reported for the first time in this family.

(8) *Tiliaceae*—*Corchorus* : The floral anatomy of seven species of *Corchorus* was studied. The earlier view that the stamen traces first trifurcate and sepal traces are independent was not supported. The placentation is anatomically axile even in the unilocular region. Stamens are considered obdiplostemonous. Two whorls

of carpels have also been reported. The embryology of *Corchorus tridens* has been described. Pollen has been reported to be tricorporate and not triplicate.

(9) *Caryophyllaceae*—*Stellaria* : The node has been considered unilacunar and opposite type of phyllotaxy as derived. The floral anatomy of six species has been studied. Sepals have been shown to be three-traced. Placentation has been found to be actually axile, but it becomes free central due to breakage of the delicate septa. Central residual vascular strand has been considered to indicate the presence of some amount of receptacular tissue. Polygonum type of embryo sac and caryophyllad type of embryo development have been reported.

(10) *Meliaceae* : The floral anatomy of members of six genera of *Meliaceae* has been reinvestigated and differences from earlier reports have been recorded. Non-vascular stamen tube teeth have been interpreted as fused or free interpetiolar stipules, placentation as axile and disc as receptacular.

(11) *Boraginaceae*—*Sericostoma pauciflorum* : Unlike earlier reports, in members of *Boraginaceae*, *Sericostoma* was observed to show only single traced sepals and unbranched carpellary dorsals.

(12) *Compositae* : The floral anatomy and embryology of five species of *Compositae* was studied and evidence was obtained for interpreting the calyculus as reduced calyx, the ovule as lateral and derived from parietal, inferior ovary as appendicular and ovary as bicarpellary. *Brachycome* was found to be an apomict. Increased number of antipodals, polygonum type of embryo sac, single layer of persisting endosperm in the fruit

and asterad type of embryo development were observed.

(13) *Papilionaceae—Trifolieae* : The study indicated that *Parochetus* included under *Trifolieae* differs from others. The sepal laterals show adnation with petal medians. Standard is not caducous.

A trilacunar three traced stipulate node has been observed in 36 species of *Trifolieae*. *Trifolium* is distinct from the other genera of the tribe in the splitting of the leaf median at the base into three and in the presence of five bundles at the base of the petiole. Trichomes of 39 species of *Trifolieae* have been worked out and their ontogeny reported.

Enough evidence was brought out in support of separation of *Ononis* from *Trifolieae* into a separate tribe *Ononideae*.

(14) *Caesalpiniaceae—Saraca* : The floral anatomy of three species of *Saraca* has been studied and the controversy regarding the outer floral whorl settled. The presence of five rudimentary petals and ten stamens in *S. cauliflora*, and the nature of the posterior sepal (fusion product of two sepals) have been confirmed from vasculature.

(15) *Cyperaceae — Fimbristylis diphylla* : The development of the female gametophyte and embryo has been described.

(16) *Commelinaceae* : The floral anatomy of 14 species representing nine genera of *Commelinaceae* was studied. The two sub-families are differentiated on the basis of supply to the outer tepals, one traced in *Tradescantieae* and three traced in *Commelinaceae*. The placentation is considered parietal derived from axile.

(17) *Dioscoreaceae—Dioscorea* : Study of the morphology, anatomy and development of bulbil in 11 species of *Dioscorea* indicated that the bulbil shows several unique characters like presence of more than one meristematic region, and tubercles from which roots or shoots are issued, etc. Study of the nodal anatomy of 20 species of *Dioscorea* revealed (a) trilacunar and a pentalacunar type (not described earlier); (b) the vascular supply to the axillary bud not supporting earlier reports of Burkill; and (c) formation of vascular plexus before the leaf supply. The floral anatomy of 11 species of *Dioscorea* was studied. Adnation of filaments with the style forming a short column was reported in *D. wallichii* resembling that of *Trichopus*. Placentation is considered parietal.

Reviews on work done on floral anatomy in India, role of floral anatomy in taxonomy and floral anatomy as one of the modern trends in taxonomy, have been published.

## (II) Morphogenesis and Tissue Culture

A review was published on vegetative to reproductive state of apex in monocotyledons. The various factors causing the profound change were discussed.

(1) *Fimbristylis diphylla* : From a study of organization of shoot apex in *Fimbristylis diphylla*, it was concluded that there is no appreciable change during the transition from vegetative to flowering state. The impulse has a spiral clock winding and, therefore, the flowers originate on a clock-wise spiral. The development of stamen and carpel has been described.



(2) *Calotropis procera*: Shoot apex organization was described. A new fourth zone comparable to the cambium-like zone of Popham and Chan was described in different phases of the plastochron and it was suggested that it may be an expression of the node in juvenile state.

(3) *Agava wightii*: Shoot apex, leaf development and unifacial tip were described. Unifacial part of the leaf has been regarded as equivalent to a phyllode and not as a sympodial structure, as suggested by Roth.

(4) *Antirrhinum majus*: Flower development, including corolla tube formation, was described. Acropetal development has been observed for all the floral organs. All organs, except stamens, are initiated in the second layer and the adjacent calyx lobes do not show post-genital fusion. Corolla tube has been considered to have been formed due to zonal growth preceded by joint growth of hump on the abaxial face of stamen primordia and interprimordial region between petal primordia.

(5) Plantlets from split anthers in nature and callusing of the staminal tissue *in vivo* in *Dianthus caryophyllus* have been observed.

### (III) Palynology

A review on cytokinesis and microspore tetrad was published, indicating the taxonomic utility of this information.

(1) *Pollen grains of Trifolieae*: Pollen grains of 34 species representing five of the six genera of Trifolieae were described. In *Medicago*, they are colpoidate and in the remaining genera, 3-zonicolporate.

### (IV) Experimental Studies

(1) *Abscission studies*: A detailed review on abscission of plant organs was published.

Several papers on abscission of cotyledonary leaf in *Gossypium hirsutum*, petiolar abscission in *Ocimum sanctum*, *Gossypium hirsutum* and *Capsicum annum*, flower abscission in *Vicia faba* and abscission of petiole and flower in these members on the application of growth substances and histochemical observations on abscission zone were published. Abscission was also found to be caused by the neighbouring surviving leaves. The abscission zone was found to be characterized by cutin deposition on epidermal cells and poor lignification in vascular elements. Leaf abscission was observed to follow the formation of a distinct abscission zone through cell division.

(2) *Plant growth studies*: The effect of various growth substances like IAA on decapitated plants of *Tagetes erecta* and 2,4,5-T on chlorophyll content of leaves of *Gossypium hirsutum* was reported.

(3) *Seedling germination and growth studies*: The effect of growth regulators on seed germination and seedling growth was studied. Increase in divergence in cotyledons of the excised embryos as an index of seed viability was described and confirmed in several members of Compositae.

(4) *Stomatal studies*: Besides describing the different types of stomata in a number of plants, an attempt has been made to describe the effect of GA, etc. on the epidermal cells of the cotyledons of *Crotalaria juncea* and stomatal characters

on the leaves of the gamma-irradiated seeds of *Brassica campestris*.

(5) *Pollen germination and pollen tube growth studies*: The effects of different growth substances on pollen germination and pollen tube growth were studied.

#### (V) Taxonomic Studies—Angiosperms

More than a dozen papers have been published on the flora, including the flora of Hastinapur; 21 new plants have been recorded from the upper Gangetic plain.

#### (VI) Taxonomy—Bryophytes

*Reboulia*, *Mannia*, *Asterella* and *Plagiochasma* were reported for the first time from Meerut district.

#### (VII) Fungi

Several pigment producing mutants have been isolated from pigmentless strains of *Fusarium solani* following exposure to ultraviolet radiation. It was observed that choline allowed pigment production.

#### Selected Publications

1. Murty Y S, Placentation in *Peperomia*, *Phytomorphology*, **2** (1952) 132-34.
2. Murty Y S, Studies in the order Piperales. II. A contribution to the study of vascular anatomy of the flower of *Peperomia*, *J Indian bot Soc*, **37** (1958) 474-91.
3. Murty Y S, Studies in the order Piperales I. A contribution to the study of vegetative anatomy of some species of *Peperomia*, *Phytomorphology*, **10** (1960) 50-59.
4. Murty Y S, Studies in the order Piperales. VIII. A contribution to the morphology of *Heuttynia cordata*, *Phytomorphology*, **10** (1960) 329-41.
5. Murty Y S & Singh V, New plant records for the upper Gangetic plain from Meerut and its neighbourhood, *Proc natn Inst Sci India*, **27** (1961) 13-17.
6. Murty Y S, Cytokinesis and microspore tetrad, in *Recent advances in palynology*, edited by P K K Nair (National Botanical Gardens, Lucknow) 1964, 176-202.
7. Murty Y S & Palser B F, Studies of floral morphology in the Ericales. VIII. Organography and vascular anatomy in *Erica*, *Bull Torrey Bot Club*, **94** (1967) 243-320.
8. Murty Y S & Kumar V, Vegetative to reproductive state of apex in monocotyledons, in *Advances in plant morphology*, edited by Y S Murty, B M Johri, H Y Mohan Ram & T M Varghese (Sarita Prakashan, Meerut) 1972.
9. Murty Y S & Prakash G, Abscission of plant organs, *Frontiers of Plant Sciences, Prof P Pariza Felicitation Volume*, 1977, 355-64.
10. Murty Y S & Gupta Sushma, Morphological studies in meliaceae. II. A reinvestigation of floral anatomy of members of Swietenieae and trichilieae, *Proc Indian Acad Sci*, **87** (1978) 55-64.
11. Murty Y S & Puri V, Floral anatomy in relation to taxonomy, in *Glimpses in Plant research*, Vol 5, *Modern Trends in Plant Taxonomy*, edited by P K K Nair (Vikas Publishing House, New Delhi) 1980, 222-43.
12. Murty Y S & Purnima, Nodal anatomy of some species of *Dioscorea* Linn, *Current Trends in Life Sciences*, Vol 9, *LP Mall's Commemoration Volume* (1980) 109-23.
13. Singh Karan & Murty Y S, Anatomy of abscission zone of experimentally retarded and accelerated leaf fall in *Capsicum annuum* L, *J Indian bot Soc*, **61** (1982) 229-33.
14. Murty Y S & Purnima, Morphology, anatomy and development of bulbil in some dioscoreas, *Proc Indian Acad Sci*, **92** (1983) 443-49.
15. Awasthi D K, Vishnu Kumar & Murty Y S, Flower development in *Antirrhinum majus* L (Scrophulariaceae) with a comment upon corolla tube formation, *Bot Mag, Tokyo*, **97** (1984) 13-22.
16. Murty Y S & Rajni Bala, Floral anatomy of *Clerodendrum*, *Current Studies in Botany, Indian bot Repr, Prof K B Deshpande Commemoration Vol*, 1984, 53-59.
17. Murty Y S, Floral anatomy in relation to taxonomy, modern trends in plant taxonomy, *Advance Notes on Symposia and Discussions, Proc Indian Sci Cong*, **72** (1985) 55-60.



## S C Pandeya

The major contributions of Pandeya to different fields of environmental science are discussed below in brief.

### (1) Structure and Functions of Grazing Lands and Forest Ecosystems

#### Grazing lands

Ecological studies on natural grazing lands in India were first initiated by him at Sagar in Madhya Pradesh in the year 1949. The work gave details of community structure, successional trends and edaphic correlation in the locality of some eight grazing land communities recognized on the basis of similarity of apparent physiognomy and depending upon topography, soil types and grazing pattern. Indeed, tropical grazing lands are seral in nature, owing their origin to deforestation or abandoned cultivation, and are so maintained at various successional levels due to anthropogenic factors.

*Growth pattern and net primary production:* Active growth of vegetation in the semi-natural grazing lands is triggered by the advent of monsoon during June/July. The live-green biomass increases to peak around September/October and then declines upon maturity of the constituent species, a process which is controlled by the climatic and edaphic factors and species themselves. It is understood that the net assimilation is

equal to the relative growth rate of the species, being the function of the magnitude of driving variables and abiotic state variables minus functions of metabolic processes. Indeed, growth in the grazing lands starts only when the actual evapotranspiration equalizes the potential evapotranspiration during June/July. Through bivariate and multivariate regression analysis, climo-edaphic vegetational relationships have been well established in the grazing lands of India. Given just mean monthly temperature and monthly rainfall, it is now possible to predict the potential net primary productivity (NPP).

*Highest NPP*—The studies have indicated that NPP is highest ( $5,000 \text{ g m}^{-2}\text{yr}^{-1}$ ) in India in Mehsana division having semi-arid ecoclimate and alluvial soils. Further, grazing lands are more productive than forest ecosystems.

A new index of water status (WS) has been evolved. It is the water budget of a place calculated from simple climatic parameters. In western India, the WS spans from  $-500$  to  $3,000 \text{ mm}$ , including forested zones of Dangs and Abu. Both total net production and total biomass, as also the ecological efficiency of energy capture, have been worked out as highly significant functions of WS. It is a trapezoid curvature with optimum values

between +500 and +1,000 mm (regime of  $C_4$ -plants). At higher WS (forested areas), the three values are lower (regime of  $C_3$ -plants).

**Impact analysis:** There is a direct inverse relationship between the degree of human activity and the organic matter productivity of the natural grazing lands in western India. Four types of economy exist in western India—forest-based, agriculture-based, bovine animal-based and bank draft-based. This is in decreasing sequence of annual rainfall. Forest-based economy exists in subhumid forested areas, agriculture-based in semi-arid alluvial flats and animal-based in the arid Great Indian Desert. Bank draft economy occurs in coastal regions and border areas from where people have gone to African countries and send back bank drafts to their families, who in turn, do not bother for the land; and also from where smuggling freely occurs. The impact has been quantified. It is apparent that overpopulation of man, his bovine animals, cutting of forest and commissioning of more land for agriculture have greatly reduced the land area for grazing resource and also highly reduced the capacity to fix solar radiations via the physical and chemical losses in soil and changes in microclimate.

#### Forest ecosystems

Working in the upper catchment area of Narmada river in the tract from Amarkantak to Mandla [sal (*Shorea robusta*) dominated forests] and there to Jabalpur [teak (*Tectona grandis*) forests], it has been shown that sal forests occur in more rainfall (humid to subhumid) and thermal regime and teak dominates in dry subhumid conditions. There is no significant role of lithology in these forest

distributions. Sal is a semi-evergreen species and teak is a deciduous one. Edaphic factors markedly differ in these forests. Based on quantitative analytic characters, some forest communities have been arrived at. A vegetational map of the classified communities has been prepared.

**Forest production:** Another important contribution from the above study was that the overall organic matter productivity and separately of wood can be measured by the non-destructive method involving simple measurement of circumference at breast height (CBH) and average tree height. The important tree species worked out in this regard are teak, sal, dhawara (*Anogeissus latifolia*), and saja (*Terminalia alata*). Optimum age at which the tree should be harvested can be worked out by superimposing tree height (hyperbola) and total biomass (parabola) curves. The curvatures meet at a point beyond which the growth is not enhanced substantially.

## (2) System Analysis and Modelling

In system context, the net primary productivity on daily basis has been predicted by preparing dynamic simulation models using differential equation and functional curvatures. The models have been validated.

Based on system approach, the consequences of implementing any perspective planning in the country's development, whether in the field of land utilization, development of a village ecosystem, forest management and afforestation programmes, industrial growth, agricultural eco-system for optimum sustained yield, erection of dam, abatement of pollution, population control, optimized herbage production and animal health, human health and efficiency or



combating of desertification, can well be understood along time scale on long-term basis and thus help to avoid environmental degradation from the very start of implementation of the programme. Optimization and not maximization is the key concept.

### **(3) Discovery and Environmental Correlations of 25 + 9 Ecotypes of two Promising Forage Species of *Cenchrus***

Extensive and intensive studies conducted on environment and *Cenchrus* grazing lands in western India have led to the discovery of 25 ecotypes of *Cenchrus ciliaris* and nine of *Cenchrus setigerus* from the arid to semi-arid regions of western India. The ecotypes are climo-edaphic ones. High forage yielding ecotypes of the two grass species complex have been established, their distributional pattern ascertained and growth behaviour found through intensive field trials. The forage plants are now being grown successfully in India and America.

### **(4) Dynamic Process of Desertification**

By making inventory and monitoring of the vectors of physical environment, including climate and soil, biological environment, including plants and animals and man and his socio-cultural heritage, it has been inferred that the rocky, sandy and saline deserts are not of recent origin. Though arid and superparched, when left to themselves, they remain green up to the extent of 40-50% of ground cover. The potential net primary production would be sufficient to sustain the entire bovine population. But it is not so allowed. Continuous and excessive use of these lands by extensive grazing (since the entire economy of the Great Indian Desert is

animal-based) has done massive harm to the otherwise fragile ecosystems by not permitting potential NPP to occur. In many cases, the status of soil and geohydrology have come almost to the point of no return.

The Great Indian Desert is prehistoric in its genesis. It is not enlarging further mainly due to the presence of geographical barriers around it.

What is more important is to undertake a programme to combat desertified areas on "self-sustained basis" by first completely stopping free grazing and then harvesting herbage at an optimized level. Compulsory stall feeding is the only solution.

The studies conducted indicate that desertification is posing greater problems in subhumid areas of central India (like Khargone, Khandwa, Rajgarh-Biora) and then in the tract adjoining the Great Indian Desert. This is mainly due to the interaction of the rocky substratum, climatic regime and the local economy (overuse of land by deforestation and overgrazing). Indeed, there is no danger of desertification in semi-arid areas where the major problem is that of land utilization pattern.

### **(5) Ecology of Some Important Species**

#### *Plant species*

Five ecological races of *Catharanthus roseus* have been isolated from western parts of India and treatments prescribed for increasing the yield of alkaloids therefrom.

Ecological adaptation to the stressed conditions of the arid zone has led to the evolution of at least five races of *Datura*,

including three new species. Further, a new species of *Solanum sysimbrifolium* has been reported for the first time from western India. The berries of the species provide the alkaloid solasodine, the yield being 3-4 times more than from any other known species in the world.

### Animal species

In western India, ecological adaptation to the hot and dry conditions has led to the evolution of at least five races of goat. In another study, it was found that the Saurashtra variety of blackbuck (Velavadar National Park) produces more of biomass and grows in numbers faster than the blackbuck variety of Kanha National Park, which is on the point of extinction.

## (6) Environment and Speciation

*A theoretical consideration* : In the case of adaptive species, gradual environmental changes bring commensurable adaptation in both physiological and morphological traits which would be heritable (ecotypes).

Under natural conditions, there is nothing like spontaneous mutation. Sudden mutation can occur only when there is some catastrophe or pollution below the lethal dose. Speciation is thus directional. Both gradual environmental change and species adaptation go side by side.

## Selected Publications

1. Pandeya S C, Grasslands of Sagar, MP, *Indian For*, **78** (12) (1952) 638-54.
2. Pandeya S C, Studies on the morphology and ecology of three species of *Dichanthium Willemet*, *J Indian bot Soc*, **32** (1953) 86-100.
3. Pandeya S C, Ecology of grasslands of Sagar, MP Pt I—Grassland map of the area on physiognomic basis, *J Indian bot Soc*, **40** (1961) 592-600; Pt IIA—Composition of fenced associations, *J Indian bot Soc*, **43** (1964) 577-605; Pt IIB—Composition of the associations open to grazing or occupying special habitats, *J Indian bot Soc*, **43** (1964) 606-39; Pt III—Edaphic factors in the distribution of the grassland associations, *Trop Ecol*, **10** (2) (1961) 163-82.
4. Pandeya S C, Dynamics of net primary productivity of grazing land and forest ecosystems in western India, *Proc First Int Cong Ecol*, The Hague, The Netherlands, 1974, 46-51.
5. Pandeya S C, Potentialities of net primary production of arid and semi-arid grazing lands of India, *Proc XII Int Grassl Cong Plenary Session*, **1** (1974) 136-70.
6. Pandeya S C, Sharma S C, Pathak S J, Jain H K, Paliwal K C & Bhanot V M, *The Environment and Cenchrus grazing lands in Western India*, US PL 480 Final Report, 1977, 451.
7. Pandeya S C, Ecology as an aid to floristics. I—Adaptation and ecotypes, *Bull bot Surv India*, **4** (1962) 141-46; II—Autecology, *Bull bot Surv India*, **4** (1962) 147-53; III—Variations in a plant population, A theoretical consideration of the dynamics of ecotype: Formation, *Symp Newer Trends in Taxonomy*, Natn Inst Sci India, 1966, **34** (1967) 240-47.
8. Pandeya S C, The environment and population differences in anjan grass (*Cenchrus ciliaris*) in Western India, *Proc XIII Int Grassl Cong*, Leipzig, **1** (1977) 31-49.
9. Pandeya S C & Jain N K, Edaphic and climatic factors in determining distribution pattern of plants in Madhya Pradesh forests, with special reference to sal (*Shorea robusta* Gaertn f), *Mem Indian bot Soc*, **3** (1960) 34-42.
10. Pandeya S C, Studies in the foliar analysis of sal (*Shorea robusta* Gaertn f) and physico-chemical status of the underlying soils with reference to Madhya Pradesh, *Bull bot Surv India*, **8** (1966) 108-16.
11. Pandeya S C, Sharma S C & Pandit B R, Dry matter production relations of sal (*Shorea robusta*) in the reserved forest communities in River Narmada upper catchment area, *J Indian bot Soc Golden Jubilee*, **50A** (1970) 601-10.
12. Pandeya S C, Pandya S M & Murthy M S, Ecology of River Narmada upper catchment area—Situation, physiography, lithology, climate and



- classification of reserved forests communities, *J Indian bot Soc*, **51** (1972) 356-73.
13. Pandeya S C & Sharma S C, Nitrogen and phosphorus status in the aboveground parts of sal (*Shorea robusta* Gaertn f) in the natural forests in River Narmada upper catchment area in Central India, *J Indian bot Soc*, **52** (1973) 51-64.
14. Pandeya S C, State of knowledge report on the grazing land ecosystems of the Indian sub-continent and Sri Lanka, *UNESCO Publ MAB Programme*, 1975, pp 359.
15. Pandeya S C, Saratbabu G V & Bhatt A B, Dynamics of solasodine accumulation in developing berries of *Solanum sysimbrifolium*, *J mdnl Pl Res*, **4** (1981) 409-11.
16. Pandeya S C, Bhatt A B & Saratbabu G V, Morphometric studies in *Datura metel* Linn, *Proc Indian Acad Sci (Plant Sci)*, **93** (6) (1984) 661-66.

## D D Pant

After doing his MSc in botany from Lucknow University in 1941, Pant started his research career under the late Prof. Birbal Sahni, FRS. He got his DPhil from Allahabad University in 1950. He joined Allahabad University as a Lecturer in 1945, becoming a Reader in 1964 and Professor in 1966. Under a British Council Grant (1954), he got the opportunity to work with Prof. T M Harris, FRS, of Reading University, Prof. John Walton of Glasgow University, Keeper W N Edwards of British Museum of Natural History, London, and Prof. R Krausal of Senckenberg Institute, Frankfurt.

Pant is known internationally for his contributions on *Glossopteris* flora, palynology, gymnosperms, stomatal development and anatomy, which provided new concepts about (1) the gametophytic nature of *Rhynia gwynnevaughani*<sup>1</sup>; (2) the nature of the primitive node and new nodal types<sup>2</sup>; (3) evolution of coniferous cone<sup>3,4</sup>; (4) evolution of the fern annulus<sup>5</sup>; (5) origin of the *Glossopteris* flora contemporaneously with the Palaeozoic ice age<sup>6</sup>; (6) Structure of the vegetative and reproductive organs of *Glossopteridales*, the reconstructions of their plants and their affinities<sup>7,8</sup>; (7) formation of asymmetrical fossil compressions<sup>9-11</sup>; and (8) new classifications of stomata<sup>12</sup>, fossil spores<sup>13</sup> and gymnosperms<sup>14</sup>.

Pant's work has led to the establishment of more than 100 new taxa, including several new genera, species and new combinations of fossil and living plants and to the recognition of a new group of gymnosperms, the *Buriadiales*. His work is cited extensively in textbooks, reviews, monographs and papers published in India and abroad. He has published over 130 original research papers and a monograph<sup>15</sup>. His work is characterized by an unusual combination of studies on fossil and living plants, often on a comparative basis.

After retirement from the professional chair in Allahabad University in 1982, Pant is continuing his work on fossil and living plants under a DST project "Studies on Indian living plants and their fossil counterparts". He has lately extended his studies to reproductive biology of fossil and living plants, including their pollination ecology. He is engaged in writing monographs on Gondwana plants and the genus *Pinus*.

Pant was invited as an expert in the first, second and third international conferences held at Tucson (Arizona), Utrecht and Novosibirsk (USSR) respectively; the first, second, third and fourth Gondwana symposia held in Argentina, France, S. Africa and Australia, respectively; and the twelfth and thirteenth International

---

Formerly Senior Professor & Head, Department of Botany, University of Allahabad, Allahabad-211002;  
Residence : 106, Tagore Tower, Allahabad-211002.



Botanical Congresses, held in Leningrad and Sydney respectively. He has been invited again to attend the international symposium on the Evolution of Gymnosperms at Montpellier in 1986 and the fourteenth International Botanical Congress in 1987 at Berlin.

Pant is a Fellow of the Indian National Science Academy, Indian Academy of Sciences, National Academy of Sciences of India, Indian Botanical Society, Linnean Society (London) and the Palaeobotanical Society. He is presently a Senior Scientist of the INSA.

### Selected Publications

1. Pant D D, The gametophyte of the Psilophytales, *Proc Summer School of Botany*, Darjeeling (1962) 276-361.
2. Pant D D & Mehra B, Nodal anatomy in retrospect. *Phytomorphology*, 14 (3) (1964) 384-87.
3. Pant D D & Nautiyal D D, On the structure of *Buriadia heterophylla* (Feistmantel) Seward & Sahni and its fructification, *Phil Trans R Soc*, 252B (1967) 27-48.
4. Pant D D, Early conifers and conifer allies, *J Indian bot Soc*, 56 (1977) 23-37.
5. Pant D D & Khare P K, *Damudopteris* gen nov. —A new genus of ferns from the Lower Gondwanas of the Raniganj Coalfield, India, *Proc R Soc*, 196B (1974) 121-35.
6. Pant D D, On two new disaccate spores from the Bacchus Marsh Tillite, Victoria (Australia), *Ann Mag Natu Hist, Lond*, 8(12) (1955) 757-64.
7. Pant D D & Singh R S, On the stem and attachment of *Glossopteris* and *Gangamoptens* leaves. Part II—Structural features, *Palaeontographica*, 147B (1974) 42-73.
8. Pant D D, The plant of *Glossopteris*, *J Indian bot Soc*, 56(1) (1977) 1-23.
9. Pant D D & Nautiyal D D, On the structure of *Raniganjia bengalensis* (Feistmantel; Rigby) with a discussion of its affinities, *Palaeontographica*, 121B (1967) 52-64.
10. Pant D D & Singh K B, Cuticular structure of some Indian Lower Gondwana species of *Glossopteris* Brongniart, Part III, *Palaeontographica*, 135B (1971) 1-40.
11. Pant D D & Bhatnagar S, Intraspecific variation in *Striaties* spores, *Palaeobotanist*, 20(3) (1971) 318-24.
12. Pant D D, On the ontogeny of stomata and other homologous structures, *Plant Sci, Ser Allahabad*, 1 (1965) 1-24.
13. Pant D D Suggestions for the classification and nomenclature of fossil spores and pollen grains, *Bot Rev*, 20 (1954) 33-60.
14. Pant D D, Population explosion in Palaeopalynology, *Proc. Symp. Structure, Nomenclature and Classification of Pollen and Spores*. Birbal Sahni Institute of Palaeobotany, Lucknow, 1971, 53-62.
15. Pant D D, The classification of gymnospermous plants, *Palaeobotanist*, 6(1957) 68-70.
16. Pant D D, *Cycas and the Cycadales* (Central Book Depot, Allahabad) 1973, i-xiv+1-255.

## V Puri

After passing MSc in botany from Agra College, Agra, in 1934, Puri joined research under Dr Panchanan Maheshwari for one year before shifting to Meerut College, Meerut, as lecturer-in-charge of the newly started Biology Department with BSc and MSc classes. In 1954, he added to it the School of Plant Morphology, with fairly good research facilities in Morphology, Taxonomy, Physiology, etc. With the help provided by the UGC and the UP Research Grants Committee, this soon became a centre of higher learning, attracting research scholars and teachers from different parts of the country and even from abroad. In 1969, Puri left, somewhat reluctantly, the Principalship of the Meerut College to join the newly created Plant Science Division in the Meerut University as Senior Professor of Botany from which position he retired three years later.

Starting his research career with the study of embryology of *Moringa*<sup>1,2</sup>, Puri soon changed over to floral anatomy at the advice of his guide. After five years' work on the orders Rhoeadales and Parietales, he got DSc degree from the Agra University in 1940. In his thesis, among other things, he supported Eames and Wilson's tetra-carpellary interpretation for the crucifer gynoecium, but suggested a different mechanism for the 'extrusion' of the ovules from the so-called 'solid

carpels', which Prof A J Eames, as one of the thesis examiners, accepted.

The early research work of Puri falls under two streams: (1) Studies in the order Parietales, wherein he studied *Tamarix*<sup>3</sup> and *Garcinia* and (2) Studies in floral anatomy. Under the second stream, he published eight papers covering besides the rhoeadalean families, Passifloraceae<sup>4,5</sup>, Cucurbitaceae<sup>6</sup>, Asclepiadaceae<sup>7</sup>, Gentianaceae<sup>8</sup>, etc. However, soon after publishing his first paper on crucifer gynoecium<sup>9</sup> in 1941, he realized that the problem of crucifer gynoecium, which is unique with its commissural stigma, parietal placentation with bilocular ovary and the placental region having two vascular bundles facing each other with the inner being inverted, —a condition also met with in several other rhoeadalean gynoecia—does not concern the number of carpels. Rather, it is a matter connected with placentation. It was, therefore, asserted that crucifer gynoecium, as a rule, comprises two carpels in the conventional sense and that the anatomical peculiarity of its placental region can be explained in terms of axile placentation. It was, therefore, suggested that the parietal placentation in most of the rhoeadalean families<sup>10</sup>, including Capparidaceae<sup>11</sup> and Moringaceae<sup>12</sup>, as also in Passifloraceae<sup>4</sup> and Cucurbitaceae<sup>6</sup> that retains the anatomy of axile

---

Emeritus Professor, Department of Botany, Meerut University, Meerut 250001; Residence: 13, Tilak Road, Meerut-250001.



placentation, may have been derived from axile placentation in the ancestral forms.

All this work prompted Puri to restate the so-called classical interpretation of the angiosperm carpel. According to him, none of the alternatives suggested so far—and they are so many—can be applied to the entire group of angiosperms with equal ease and conviction. The classical interpretation, as envisaged by him, still serves as a good working hypothesis<sup>13</sup>.

In his Presidential Address delivered at the Annual Conference of the Society for Advancement of Botany at Ludhiana, Puri drew attention to some important peculiarities of the angiosperm carpel. Some of these, due to lack of proper understanding, were cited in the past to invalidate the classical interpretation. Puri, however, has interpreted them all in terms of the classical concept<sup>14</sup> and has thereby strengthened it further.

During 1949-50, while working as a visiting scientist at the Cornell University, New York, Puri published two exhaustive review articles: (1) Role of floral anatomy in the solution of morphological problems<sup>15</sup>, and (2) Placentation in Angiosperms<sup>16</sup>, in *Botanical Review* in 1951 and 1952 respectively. In the first of these, it was emphasized on the basis of a critical review of the work available till then, that while we cannot ignore floral anatomical work in a discussion of morphological problems, we shall be equally unfair to it if we claim too much for it in this connection, as has been done by some authors.

In the article on placentation, he discussed the various types of placentation, their terminology and attempted to give an anatomical basis for

distinguishing them. Evolutionary trends in placentation were also discussed. As to the age-old controversy of the ovules being axial or appendicular, it was pointed out that in the present state of our knowledge this has lost much of its significance and attraction, especially in the light of such concepts as the partial shoot hypothesis or the telome hypothesis, the distinction between axis and leaf being no longer fundamental.

A little later Puri also reviewed the work on the angiosperm ovule in his address to the Botany Section of the Indian Science Congress Session at Kharagpur in 1970<sup>17</sup>. He concluded here that the angiosperm ovule has possibly arisen independently of the gymnosperm ovule. The two have no direct connection and hence should not be homologized with each other.

Besides the rhoeadalean families, Puri has also made a detailed study of the problem of placentation in a number of interesting families, e.g. Passifloraceae<sup>4</sup>, Cucurbitaceae<sup>6</sup>, Asclepiadaceae<sup>7</sup>, Gentianaceae<sup>8</sup>, etc. All this has enabled him to have a special insight into the problem of placentation in angiosperms.

Another review article pertains to the history of botanical research in India, Burma and Ceylon. In early fifties, the Indian Botanical Society, with the encouragement of UNESCO, decided to bring out articles on this subject. Puri was entrusted the task of writing the floral anatomy part of it and this was published by the Society as Part IV in 1961<sup>18</sup>.

Puri has also made a detailed study of the corona in the flowers of Passifloraceae<sup>5</sup>, Asclepiadaceae<sup>7</sup> and other accessory floral organs<sup>19</sup>, such as epicalyx, involucre, cupule, nectary, disc, etc. While the corona in Passifloraceae and

Asclepiadaceae is interpreted as petaline and staminal respectively, the homology of nectaries is described as most controversial for the simple reason that they are most variable in form, structure and position. The structure of the cupule in some of the Amentiferae has also been analysed and it has been concluded that while it is very variable in the Fagaceae, it is bracteal in nature in the Juglandaceae.

One field in which the role of floral anatomy appears to have been over-emphasized is that of the inferior ovary. On the basis of the course of the vascular bundles, the outer portion of the wall of the inferior ovary has very often been interpreted as appendicular, being formed by the fusion of the basal parts of the outer floral organs. A critical evaluation of all such works has revealed that in many cases floral anatomy cannot solve this problem. Every vascular bundle has dual nature; it is a *trace* as long as it traverses the receptacle or axis and becomes a *bundle* when it traverses an organ. Puri has argued that as we cannot distinguish as to where the trace ends and the bundle begins, we cannot determine, on the basis of floral anatomy, the limits between the axis and its appendages if these at all exist<sup>20</sup>.

Puri has also published some papers on the role of floral anatomy in the solution of taxonomic problems<sup>21</sup>, and on taxonomy and phylogeny<sup>22</sup>. It is well-known that vasculature of floral organs shows great deal of variations, particularly with respect to reduction and amplification in the number of bundles and their cohesion and adnation. These also bring about corresponding changes in taxonomic characters. And sometimes what is not visible externally may be revealed by a

study of its vasculature simply because this is not exposed to the same spatial pressure that operates on the organs that contain it.

Regarding determination of the phylogeny of plants or plant groups, Puri has emphasized that in the absence of any concrete evidence it is very difficult, if not impossible. The issue is further complicated by such processes as parallelism, convergence, inter-breeding and hybridization.

In a few joint papers, attention was focussed on anatomical and developmental aspects of *Dalbergia* shoot apex<sup>23</sup> and on some Pteridophytes. On the basis of a study of the anatomy of sporocarp of *Marsilea minuta*<sup>24</sup> and a critical appraisal of the work reported earlier, it was concluded that the sporocarp of *Marsilea* is equivalent to a single leaflet which consisted of as many pinnules as the number of commissural bundles that form the mid-rib of the former. The bilobed condition of the fertile pinnules, which is an essential feature of the interpretation put forward here, offers a satisfactory explanation for the location of the sorus in the plane of the commissural bundles. In another investigation<sup>25</sup>, the shoot apex organization in *Selaginella*, *Lycopodium* and *Isoetes* was studied. It has been emphasized that there is no uniformity in organization and structure of the shoot apex in these taxa. In a more recent publication<sup>26</sup>, the work done so far on the root apex and shoot apex organization in the pteridophytes has been reviewed critically and in all four different types of root and three of shoot apical configurations are recognized and discussed.



In the Sixth Annual Lecture of the Palynological Society of India, Puri referred to some aspects of the structure and function of the anther sacs and pollen grains<sup>27</sup>. He emphasized that angiosperm organs should be studied on the basis of an "angiosperm centered approach"; their interpretation in terms of other plant group without any phylogenetic evidence may lead to a good deal of confusion. He has also warned against seeking strict homologies between stamen and carpel.

During the last three decades or so we have been hearing a great deal of the so-called "new morphology" that is essentially historical or phylogenetic in approach. This is intended to be an alternative to the classical approach which is essentially idealistic. Puri has examined this issue in some detail and concludes that these two approaches are not antagonistic to, or exclusive of, each other and that they can coexist if they are valid in their own spheres<sup>28,29</sup>.

In a couple of other publications, Puri has analysed the issues involving the relationships of leaf and stem<sup>30</sup> and ovule and carpel<sup>31</sup>. It has been emphasized herein that these are man-made categories created to serve convenience of description rather than anything else and these must be so recognized.

Among the many book reviews that Puri published in various journals, those covering Agnes Arber's *Philosophy of the Plant Form* and *The Mind and the Eye*, A Takhtajan's *Die Evolution der Angiospermen*, M Aberchrombie & J Brachet's *Advances in Morphogenesis*, Vol 1, K R Sporne's *Pteridophytes*, I W

Bailey's *Contributions to Plant Anatomy* and Esau's *Anatomy of Seed Plants* are worth mentioning.

Some papers on general topics such as the concept of carpellary margins<sup>32</sup>, salient concepts in plant morphology<sup>33</sup>, basic assumptions in plant morphology,<sup>34</sup> the so-called synthetic approach to plant morphology<sup>35</sup>, etc. have also been published.

Lately, Puri has become interested in the problem of origin and evolution of angiosperms. A general account of the subject was presented in the form of his Presidential Address to the Indian Botanical Society at Bangalore<sup>36</sup>. In the Seventh Silver Jubilee Lecture, delivered at and published by the Birbal Sahni Institute of Palaeobotany, Lucknow, he analysed in detail the issues of the so-called primitive angiosperms. Subsequently, he has dealt with the habit and habitat of early angiosperms<sup>37</sup> and whether they are monophyletic or polyphyletic in origin<sup>38</sup>. Other relevant questions like: (1) Where did they originate? (2) What were their immediate ancestors? (3) How are the monocots related to dicots?, etc. are engaging his attention.

In a wider context, more significant perhaps than all the research work of Puri is his contribution to the development of botany at Meerut. Within less than 20 years of his joining the Meerut College, he could establish teaching facilities in botany up to the highest standard and create fairly good research facilities in different areas of research at Meerut, where none existed before. These facilities have been widely used by students of the region as well as by scholars from different parts of the country and also from abroad.

## Selected Publications

1. Puri V, Embryo sac and embryo of *Moringa oleifera* Lamk., *Nature, Lond*, **135** (1935) 70.
2. Puri V, The life history of *Moringa oleifera* Lamk., *J Indian bot Soc*, **20** (1941) 263-84.
3. Puri V, Studies in the order Parietales. 1. A contribution to the morphology of *Tamarix chinensis* Lour, *Beih Bot Zbl*, **A59** (1939) 335-49.
4. Puri V, Studies in floral anatomy. IV, Vascular anatomy of the flower of certain species of the Passifloraceae, *Am J Bot*, **34** (1947) 562-73.
5. Puri V, Studies in floral anatomy. V, On the structure and nature of corona in certain species of the Passifloraceae, *J Indian bot Soc*, **27** (1948) 130-48.
6. Puri V, Studies in floral anatomy. VII, Placentation in certain species of the Cucurbitaceae, *Phytomorphology*, **4** (1954) 278-99.
7. Puri V & Shiam R, Studies in floral anatomy. VIII, Vascular anatomy of the flower of certain species of the Asclepiadaceae with special reference to corona, *Agra Univ J Res (Sci)*, **15** (1967) 189-16.
8. Puri V & Gopal Krishna, Morphology of the flower of some Gentianaceae with special reference to placentation, *Bot Gaz*, **124** (1962) 42-57.
9. Puri V, Studies in floral anatomy, Gynoecium constitution in the Cruciferae, *Proc Indian Acad Sci*, **14B** (1941) 166-87.
10. Puri V, Studies in floral anatomy. III, On the origin and orientation of placental strands, *Proc natn Acad Sci, India*, **15** (1945) 74-91.
11. Puri V, Studies in floral anatomy. VI, Vascular anatomy of flower of *Crataeva religiosa* with special reference to the nature of carpels in the Capparidaceae, *Am J Bot*, **37** (1960) 363-70.
12. Puri V. Studies in floral anatomy. II, Floral anatomy of the Moringaceae with special reference to gynoecium constitution, *Proc natn Inst Sci India*, **8** (1942) 71-88.
13. Puri V, The classical concept of angiosperm carpel: A reassessment, *J Indian bot Soc*, **40** (1961) 511-24.
14. Puri V, On some peculiarities of angiosperm carpel, *Acta Bot Indica*, **6** (Suppl) (1978) i-xiv.
15. Puri V, The role of floral anatomy in the solution of morphological problems, *Bot Rev*, **17** (1951) 471-553.
16. Puri V, Placentation in angiosperms, *Bot Rev*, **18** (1952) 603-51.
17. Puri V, The angiosperm ovule, Presidential Address, *Botany Section, Indian Sci Cong Session* (1970) 1-36.
18. Puri V, History of botanical researches in India, Burma & Ceylon. Part IV—*Indian bot Soc* (1961) 1-31.
19. Puri V & Agarwal R M, On accessory floral organs, *J Indian bot Soc*, **55** (1976) 95-114.
20. Puri V, Floral anatomy and inferior ovary, *Phytomorphology*, **2** (1952) 122-29.
21. Puri V, Floral anatomy in relation to taxonomy, *Bull bot Surv India*, **4** (1962) 161-65.
22. Puri V, Classification and phylogeny, *Bull bot Surv India*, **4** (1962) 167-72.
23. Agarwal R M & Puri V, Ontogenetic studies in some important trees of India. 1, Shoot apex organization and leaf development in *Dalbergia sissoo*, *Phytomorphology*, **27** (1977) 296-302.
24. Puri V & Garg M L, A contribution to the anatomy of the sporocarp of *Marsilea minuta* L. with a discussion of the nature of sporocarp in Marsileaceae, *Phytomorphology*, **3** (1953) 190-209.
25. Bhambie S & Puri V, Shoot apex organization in Lycopodiales, *Indian bot Soc Memo*, **4** (1963) 55-61.
26. Bhambie S & Puri V, Shoot and root apex meristems, *Trends in Pl Res*, edited by C M Govil & V Kumar (1985) 55-81.
27. Puri V, Anther sacs and pollen grains: Some aspects of their structure and function, 6th Annual Lec Palynol Soc India, *J Palynol*, **6** (1970) 1-17.
28. Puri V, Disappointments of a morphologist, *J Indian bot Soc*, **44** (1965) 7-13.
29. Puri V, Morphology "old" and "new" and the evolution of flower, *J Indian bot Soc*, **54** (1975) 131-38.
30. Puri V, On the relation between leaf and stem, *Bull bot Soc Bengal*, **8** (1954) 101-8.
31. Puri V, On the relation between ovule and carpel, *J Indian bot Soc, Maheshwari Comm. Vol*, **42A** (1964) 189-98.
32. Puri V, On the concept of carpellary margins, *Proc Summ Sch Bot*, edited by P Maheshwari et al (1962) 326-33.
33. Puri V, On some more salient concepts in plant morphology, *From Strc Func, B M Johri Comm Vol*, (1975) 369-74.



34. Puri V, On some basic assumptions in plant morphology, *J Indian bot Soc Golden Jubilee Vol*, **50A** (1971) 196-202.
35. Puri V, On the so-called synthetic approach in plant morphology, *Rec Trends & Contacts between Cytogen, Emb Morph*, edited by V R Dnyansagar (1977) 463-68.
36. Puri V, The origin and evolution of angiosperms, *J Indian bot Soc*, **46** (1967) 1-14.
37. Puri V, Habit and habitat of early angiosperms, *Contemp Trends Pl Sci*, edited by S C Verma (1981) 64-71.
38. Puri V, Origin of angiosperms: Monophyletic or polyphletic?, *Rec Devel Pl Sci*, edited by S P Sen (1982) 33-42.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

## M B Raizada

Raizada is well known for his outstanding contributions in Indian taxonomy and agrostology. He has done significant work on the exploration, identification and naming of a large number of plants and grasses in the Indo-Gangetic plains and the western Himalayas.

His contribution to the taxonomy of the grasses of Gangetic plains represents the first consolidated account in this respect. He is among the few who have studied the Indian Bambuseae. He has revised the Bambuseae of British India, originally written by Gamble. This work was assigned to him by the Council of Scientific and Industrial Research under the advice of Prof. P Maheshwari, FRS.

Raizada has to his credit over 200 papers published in various national and international journals and eight books. His proposal entitled "New difficulties facing plant taxonomists" put up at the Paris Congress was published in *Taxon* in 1953.

As a member of the team from South East Asia, under the Colombo Plan, he visited a number of herbaria and gardens in Europe and UK.

Raizada has carried out explorations in the NW Himalayas and made extensive collections in Tamil Nadu, Gujarat, Bengal, Assam and UP. His collections, in the form of about 100,000 specimens of angiosperms and ferns, are preserved in the Dehradun herbarium, their duplicates having been sent to the Kew and Edinburgh herbaria and some other national and international herbaria.

Raizada has played a significant role in the organization of education in forest botany in the country.

Raizada is the Honorary Editor of the quarterly "*Indian Journal of Forestry*".

Raizada retired as Chief Research Officer and Head, Division of Botany, Forest Research Institute and Colleges, Dehradun.

### Selected Publications

1. Raizada M B, *Beautiful climbers and shrubs*.
2. Raizada M B, *Grasses of Upper Gangetic Plain*.
3. Raizada M B, *Living Indian gymnosperms*.
4. Raizada M B, *Flora of Gir Forest*.
5. Raizada M B, *Flora of Mussoorie*.
6. Raizada M B, *Essentials of forest botany*.
7. Raizada M B, *Flora of Andhra*.
8. Raizada M B, *Trees for Van Mahotsava*.

---

Formerly, Chief Research Officer & Head, Division of Botany and Professor Emeritus, Forest Research Institute & Colleges, Dehradun; Residence : 25-C, Circular Road, Dehradun.



## V S Rama Das

The main thrust of research work of Rama Das has been in the area of photosynthesis, with special reference to plant productivity. This has included stomatal and non-stomatal parameters in the control of photosynthesis,  $C_4$  photosynthesis and the herbicide action in photosynthesis. His current research interests include the analysis of photosynthesis in the naturally occurring  $C_3/C_4$  intermediates, regulation of electron transport and membrane polypeptide composition in relation to environmental variables, light and temperature. The use of isolated leaf cells and of protoplasts for organelle preparation and the study of distribution of photosynthetic and photo-respiratory enzymes is another current approach.

### **Stomatal Component: Mechanisms of Regulation and Antitranspirant Action**

The role of light in stomatal opening was established with the demonstration of the involvement of cyclic photophosphorylation. The antitranspirant properties of cyclic photophosphorylation inhibitors were observed for the first time; these indicated the possibility of a field scale application of these compounds as potential antitranspirants for crop plants. The use of antitranspirants for achievement of higher water use efficiency by several crops was also indicated.

The guard cell chloroplasts of *Commelina benghalensis* were isolated for the first time and their high photochemical potential was determined; the results supported the view of the dependence of stomatal opening on cyclic photophosphorylation. The enhancement of stomatal aperture by exogenous pyruvate has confirmed the active role of organic acids as balancing anions in stomatal opening. Studies on enzymes in guard cells have revealed that phosphoenolpyruvate carboxylation was the basis for malate production in guard cells and that ATP was necessary for the conversion of pyruvate into phosphoenolpyruvate. Based on the above findings, a mechanism has been proposed to explain stomatal opening and its control by light, ATP, potassium ions and carbon dioxide. The observation that isoenzymes of ATPase are associated with stomatal movement is an important original finding. These findings have opened up new vistas in knowledge on stomatal physiology.

Leaf gas exchange measurements have shown that stomatal conductance for  $CO_2$  is well correlated with net photosynthesis in wheat cultivars.

### **$C_3$ and $C_4$ Photosynthesis**

The occurrence of  $C_4$  photosynthesis was reported in many mono- and dicotyledonous plants and the natural

---

Professor and Chairman, Department of Botany, and Dean, Faculty of Science, Sri Venkateswara University, Tirupati-517502; Residence : No. 2 Red Buildings, S. V. University Campus, Tirupati-517502.

existence of interspecific differences in some of the genera was reported in species hitherto not reported. A detailed study of the physiology and biochemistry of photosynthesis of some selected  $C_4$  mono- and dicotyledonous species was conducted.  $C_4$  plants were found to exhibit considerable diversity in their photosynthetic carbon metabolism; they have been classified into three groups based on their anatomical, biochemical and biophysical features. Purification of phosphoenolpyruvate carboxylase, the key enzyme in  $C_4$  photosynthesis, from a variety of  $C_4$  plants, has been achieved. The inhibition of phosphoenolpyruvate carboxylase by malonate is a new finding. It was confirmed that oxaloacetate and malate were potential inhibitors of the enzyme.

Studies on the distribution of the photosynthetic and photorespiratory enzymes in mesophyll and bundle sheath cells and chloroplasts have improved our understanding of the highly coordinated photochemical and biochemical mechanisms functioning in the two cell types of  $C_4$  plants. They have also led to a better understanding of comparative physiology of  $C_3$  and  $C_4$  photosynthesis.

Presently, the naturally occurring species intermediate between  $C_3$  and  $C_4$  plants in the genus *Alternanthera* which were hitherto not known have been reported. The intermediate nature of these species is based on leaf anatomy,  $CO_2$  compensation point,  $O_2$  response to  $CO_2$  compensation point, photosynthetic rate and the biochemistry of photosynthesis. Studies on *Mullugo nudicaulis* showed some leaves with  $C_3$  characteristics and others with  $C_4$  characteristics according to their position

on the stem. The occurrence of kranz-like leaf anatomy, a low photorespiratory activity, and high levels of phosphoenolpyruvate carboxylase in *Parthenium hysterophorus*, were observed recently and the significance of a low photorespiratory activity for the luxurious growth of this species was pointed out.

Contributions on the role of light intensity in photosynthesis and plant productivity of some tropical crops have led to the conclusion that it influences both the structural and functional relationships of chloroplasts. The activities of photosystem I and even more so of photosystem II were lowered in plants grown under low light stress. At a high light intensity, changes in the amount of cytochrome *f* had a close relationship with levels of P700, but at low light intensities, the amounts of electron carriers and the reaction centre are independent. A photoacoustic spectroscopic technique has been made use of in the interpretation of pigmentation of leaves in relation to soil moisture and adaptation to arid environment.

Diaheliotropic solar tracking leaf movements were observed in some dicotyledonous species; this is an important finding, as they have been shown to occur irrespective of the photosynthetic type. The bioproductivity of such plant species was investigated and it was found that the solar tracking ability is more efficient compared to that in closely related non-tracking species.

Measurements of biomass yields from selected  $C_3$  and  $C_4$  plants showed that  $C_4$  plants gave distinctly higher yields than  $C_3$  species. The higher biomass yields of  $C_4$  plants were related to higher carboxylation



efficiency and lack of manifestation of photorespiration in these plants.

### Leaf Cells and Protoplasts

A technique for leaf cell isolation by mechanical grinding with a suitable osmoticum was developed and used to test a large number of higher plants that have yielded intact and photosynthetically active mesophyll cells. The high frequency of cell release in some of the plants was invariably correlated with the high percentage of leaf chlorophyll recovery from these cells. These intact cells could mediate diverse metabolic reactions and were, therefore, found useful for comparative metabolic studies under a variety of conditions.

The isolation of mesophyll and bundle sheath protoplasts by enzymatic digestion and differential centrifugation from a  $C_4$  dicot species has been achieved. These protoplasts are being used for the isolation of intact chloroplast and for investigations on the distribution of photosynthetic enzymes between cell types.

### Herbicides

The possible use of herbicides to control the unproductive semi-arid scrub species was investigated. Photochemical and biochemical reactions of photosynthesis in some of the scrub species under the influence of herbicides were investigated to understand the mechanism of action of herbicides with special reference to photosynthesis. Two herbicides, paraquat and 2,4,5-T, caused an apparent reversal of the adaptive CAM-syndrome in some scrub species, resulting in desiccation of the foliage through induction of day-time stomatal opening leading to excessive transpiration.

### Role of Micronutrients

The role of zinc in photosynthesis was investigated in rice and pearl millet. Zinc deficiency was found to enhance photorespiration in rice, as evidenced by an increase in  $CO_2$  compensation concentration and the levels of photorespiratory enzymes. It is believed that the lowered carbonic anhydrase activity results in the enhancement of photorespiration in rice.

### Selected Publications

1. Das V S R & Raghavendra A S, Role of cyclic photophosphorylation in the control of stomatal opening, in *Mechanism of Regulation of Plant Growth*, edited by R L Bielecki, A R Ferguson and M M Cresswell, *Bulletin 12, The Royal Society of New Zealand, Wellington*, 1974, 455-60.
2. Das V S R & Rathnam C K M, An exclusively aspartate forming  $C_4$ -photosynthesis in *Eleusine coracana* Gaertn, in *Mechanism of Regulation of Plant Growth*, edited by R L Bielecki, A R Ferguson and M M Cresswell, *Bulletin 12, The Royal Society of New Zealand, Wellington*, 1974, 223-28.
3. Raghavendra A S & Das V S R, Distribution of  $C_4$  dicarboxylic acid pathway of photosynthesis in local monocotyledonous plants and its taxonomic significance, *New Phytol*, **76** (1976) 310-5.
4. Madhusudana Rao I, Swamy P M & Das V S R, The reversal of scotoactive stomatal behaviour in some woody weeds by paraquat and 2,4,5-T, *Weed Sci*, **25** (1977) 469-72.
5. Das V S R & Santhakumari M, The incomplete evolution of  $C_4$  photosynthesis within the pantropical taxon, *Boerhaavia* (Nyctaginaceae), *Photosynthetica*, **12** (1978) 418-22.
6. Raghavendra A S & Das V S R, Photochemical characteristics of mesophyll and bundle sheath chloroplasts from  $C_4$  plants, *Physiol Pl*, **43** (1978) 107-13.
7. Raghavendra A S & Das V S R, Photosynthetic carbon metabolism in leaves of  $C_4$  and  $C_3$  plants: A detailed comparative study, *Z. Pflanzenphysiol*, **87** (1978) 297-311.

8. Raghavendra A S, Rajendrudu G & Das V S R, Simultaneous occurrence of  $C_3$  and  $C_4$  photosynthesis in relation to leaf position in *Mollugo nudicaulis*, *Nature, Lond*, **273** (1978) 143-44.
9. Das V S R & Raghavendra A S, Antitranspirants: Improvement of water use efficiency of crops, *Outlook Agric*, **10** (1978) 92-98.
10. Raghavendra A S & Das V S R, The occurrence of  $C_4$  photosynthesis: A supplementary list of  $C_4$  plants reported during late 1974-mid 1977, *Photosynthetica*, **12** (1978) 200-8.
11. Rajendrudu G, Madhusudana Rao I, Raghavendra A S & Rama Das V S, Isolation of intact mesophyll cells from the leaves of higher plants, *Proc Indian Acad Sci*, **88** (1979) 143-54.
12. Rajendrudu G & Rama Das V S, Solar tracking and light interception in leaves of some dicot species, *Curr Sci*, **50** (1981) 618-20.
13. Rajendrudu G & Das V S R,  $C_4$  photosynthetic carbon metabolism in leaves of aromatic tropical grasses—Leaf anatomy,  $CO_2$  compensation point and  $CO_2$  assimilation, *Photosynth Res*, **2** (1981) 225-33.
14. Das V S R, Veeranjanyulu K & Ramachandra Reddy A, Photosynthesis and bioproductivity in some crop and weed species, in *Photosynthesis VI, Photosynthesis and Productivity, Photosynthesis and Environment*, edited by G Akoyunoglou (Balaban International Science Service, Philadelphia), 1981, 63-72.
15. Rajendrudu G & Rama Das V S, *Parthenium hysterophorus* L. (Asteraceae) exhibiting low photorespiration, *Curr Sci*, **50** (1981) 592-93.
16. Rajendrudu G & Rama Das V S, The carboxylating enzymes in leaves of *Cleome gynandra*, a  $C_4$  dicot plant, *Pl Sci Lett*, **26** (1982) 285-91.
17. Venkataramana S & Das V S R, Distribution of nitrogen assimilating enzymes in relation to photosynthesis in certain  $C_4$  grasses, *Z. Pflanzenphysiol*, **105** (1982) 289-96.
18. Rajendrudu G & Rama Das V S, Biomass production of two species of *Cleome* exhibiting  $C_3$  and  $C_4$  photosynthesis, *Biomass*, **2** (1982) 223-27.
19. Chenga Reddy V, Raghavendra A S & Das V S R, Photosynthetic units and carbon assimilation in leaves of grain *Sorghum* under different light intensities, *Pl Cell Physiol*, **24** (1983) 1395-1400.
20. Rao A N, Seethambaram Y & Rama Das V S, Growth and productivity of three *Sorghum* cultivars under low light stress, *Indian J exp Biol*, **22** (1984) 260-66.
21. Chenga Reddy V, Bhaskar C V S, Raghavendra A S & Das V S R, Photosynthesis of wheat cultivars in relation to photosynthetic unit and stomatal conductance, *Photosynthetica*, **18** (1984) 226-30.
22. Murthy B V, Raghavendra A S & Rama Das V S, Stomatal opening in isolated epidermis of *Commelina benghalensis* L. heterophasic response to KCl concentration, *Pl Cell Rept*, **3** (1984) 199-202.
23. David Jaya Kumar U, Saraswathy R & Rama Das V S, Differential performance of *Cleome gynandra* L. ( $C_4$ ) and *C. speciosa* L. ( $C_3$ ) under water stress and recovery, *Environ exp Bot*, **24** (1984) 305-10.
24. Sathambaram Y, Rao A N & Das V S R, The levels of carbonic anhydrase and of photorespiratory enzymes under zinc deficiency in *Oryza sativa* L. and *Pennisetum americanum* L. Leeke, *Biochem Physiol Pflanzen*, **180** (1985) 107-13.
25. Rajendrudu G, Prasad J S R & Rama Das V S,  $C_3$ - $C_4$  intermediate species in *Alternanthera* (Amaranthaceae): Leaf anatomy,  $CO_2$  compensation point, net  $CO_2$  exchange and activities of photosynthetic enzymes. *Plant Physiol*, **80** (1986) 409-14.



## P S Ramakrishnan

The three distinct phases in the development of ecological studies conducted by Ramakrishnan are at the levels of (i) individual, (ii) population, and (iii) community and ecosystem.

### Individual Ecology

These studies, initiated in 1957, are autoecological in nature and concern a number of weeds of the upper Gangetic plains. They are related to adaptation and microdistribution at the species and sub-specific levels. At the specific level, studies on light and nutritional interaction in the distribution of *Peristrophe bicalyculata* and on edaphic adaptation of *Argemone* species or aquatic weeds are important.

The recognition of edaphic ecotypes in a large number of species, starting with the work on *Euphorbia thymifolia*, is a pioneering effort at the level of adaptation of populations at the sub-specific level, and indicates a level of differentiation and micro-distribution besides that based on climate and geography. The demonstration of the existence of edaphic sub-types within edaphic ecotypes, as in *Euphorbia thymifolia*, indicates that adaptive differentiation operates in relation to the whole pattern of selective forces in the environmental complex.

An in-depth study was done on edaphic ecotypes, but with reference to the calcicole-calcifuge problem in plants. The

adaptation of plant populations to varied calcium levels was related to calcium concentration in the soil itself and to excessive availability/short supply of the nutrients, such as N, P, K, H, Al and Mn. Studies on edaphic ecotypes were also done in relation to salt tolerance in saline-alkali soils and it was shown that salt tolerant populations within species often have a higher salt level in plant tissues before toxicity is manifested. In all these studies, inter-ecotypic competition was shown to exaggerate differences between ecotypes and to play an important role in their restriction. Ecotype differences in most of these studies were shown to occur at very short distances, often less than a metre. The work on *Chenopodium album* populations was related to their distribution in space as related to seasonal differences in temperature and photoperiod.

### Population Ecology

The studies on plant population biology were initiated with work on weed-crop competitive interactions in both pure and mixed stands of varied proportions. The growth rate of species was shown to be an important attribute determining population behaviour, apart from ecological amplitude and preferences, and genetic make up of the population.

The studies on competition led to a consideration of co-existence of closely related species, such as that of *Argemone mexicana* and *A. ochroleuca* in the same area. Apart from competitive differences related to species characteristics or those related to environmental factors, the differential requirements of the resources of the environment determine the co-existence of species, as was shown for *Argemone* species or for populations of *Chenopodium album*.

### Community and Ecosystem Analysis

During the last 11 years, he has been involved in a comprehensive whole system analysis of shifting agriculture (locally known as 'jhum') and its impact on the environment. This practice essentially involves slash and burn of natural vegetation, such as a forest, followed by chopping for a year or two, abandoning the plot for fallow regrowth, before coming back to the plot after a given time period (one jhum cycle).

The study of the agricultural system is related to cropping and yield patterns, photosynthetic efficiency, nutrient uptake and the use efficiencies of different species, recycling of resources, energy budget analysis and weed potential under different jhum cycles. The 'non-weed' concept, where a weed species below a given density level has a conservation role in the agro-ecosystem, has also been studied.

During slash and burn and cropping phases, the perturbations on the ecosystem are severe. These are related to hydrology and associated losses of nutrients, soil fertility depletion, biomass and productivity changes in the ecosystem and drastic changes in the nutrient cycling

pattern. All these functional aspects of the process of ecosystem development and stabilization were followed in some cases up to 50 years of forest regrowth and further compared with those for more mature forest types. When short jhum cycles are successively imposed on the same site, desertification sets in. This phenomenon was studied at different sites, including Cherrapunji.

During detailed analysis of the successional process, the ecophysiological attributes of developing communities, including herbaceous weeds, shrubs and trees, were considered along with extensive demographic analysis. These were related to relative growth rate, net assimilation rate, allocation pattern of biomass and nutrients, nutrient uptake and use efficiencies and  $C_3/C_4$  strategies through succession of plant communities.

An aspect studied by us in a comprehensive fashion for the first time anywhere was the growth strategy and architecture of trees. The architectural design of a tree comes through a process of natural selection and is aimed at optimizing photosynthesis under a given light regime in the forest. Extension growth of the leader axis and branches, production pattern and organization of branches of different orders, branch orientation and biomass allocation patterns were some of the aspects considered here. Leaf as a meta-population on a tree was analysed for birth and death rates, leaf life-span and turnover rates. These attributes were related to shade tolerance/intolerance of trees over a successional gradient and the ability of the species to maximize production under exploitative and competitive environments in a forest. These studies have applications



in agro-forestry, social forestry and quicker rehabilitation of damaged/desertified sites.

Through a series of studies on the functioning of the village ecosystem, considering land use, animal husbandry and domestic systems under varied socio-economic and socio-cultural conditions of the tribals of the north-east, location specific eco-development strategies have been worked out. The tribal development strategy suggested for the hill regions of the north-east is based on a value system that is part of the tribal society and which they can identify themselves.

### Selected Publications

1. Ramakrishnan P S, Studies on edaphic ecotypes in *Euphorbia thymifolia* L. II, Growth performance, mineral uptake and inter-ecotypic competition, *J Ecol*, **53** (1965) 705-14.
2. Ramakrishnan P S, Nutritional requirements of the edaphic ecotypes in *Melilotus alba* Medic. III, Interference between the calcareous and acidic populations in the two soil types, *New Phytol*, **69** (1970) 81-86.
3. Ramakrishnan P S & Kumar S, Productivity and plasticity of wheat and *Cynodon dactylon* (L) Pers in pure and mixed stands, *J appl Ecol*, **8** (1971) 85-98.
4. Ramakrishnan P S & Gupta V, Nutrient factors influencing the distribution of two closely related species of *Argemone*, *Weed Res*, **12** (1972) 234-40.
5. Ramakrishnan P S & Nagpal R, Adaptation to excess salts in an alkaline soil population of *Cynodon dactylon* (L) Pers, *J Ecol*, **61** (1973) 369-81.
6. Ramakrishnan P S & Jeet N, Competitive relationship existing between two closely related species of *Argemone* living in the same area, *Oecologia (Berl)*, **9** (1972) 279-88.
7. Ramakrishnan P S & Kapoor P, Photoperiodic requirements of seasonal populations of *Chenopodium album* L, *J Ecol*, **62** (1974) 67-73.
8. Toky O P & Ramakrishnan P S, Cropping and yields in agricultural systems of the north-eastern hill region of India, *Agro-Ecosystems*, **7** (1981) 11-25.
9. Ramakrishnan P S & Toky O F, Nutrient status of hill agro-ecosystems and recovery pattern after slash and burn agriculture (Jhum) in north-eastern India, *Pl Soil*, **60** (1981) 41-64.
10. Ramakrishnan P S & Mishra B K, Population dynamics of *Eupatorium adenophorum* Spreng during secondary succession after slash and burn agriculture (Jhum) in north eastern India, *Weed Res*, **22** (1981) 77-84.
11. Singh J & Ramakrishnan P S, Structure and functions of a sub-tropical humid forest of Meghalaya I, II, and II, *Proc Indian Acad Sci (Pl Sci)*, **91** (1982) 241-80.
12. Mishra B K & Ramakrishnan P S, Energy flow through a village ecosystem with slash and burn agriculture in north-eastern India, *Agric Syst*, **9** (1982) 57-72.
13. Ramakrishnan P S, Shukla R P & Boojh R, Growth strategies of trees and their application to forest management, *Curr Sci*, **51** (1982) 448-55.
14. Saxena K G & Ramakrishnan P S, Partitioning of biomass and nutrients in the secondary successional herbaceous populations subsequent to slash and burn, *Proc Indian natn Sci Acad*, **B48** (1982) 807-18.
15. Mishra B K, Slash and burn agriculture at higher elevations in north-eastern India. I. Sediment, water nutrient losses, *Agric Ecosyst Environ*, **9** (1983) 69-82.
16. Toky O P & Ramakrishnan P S, Secondary succession following slash and burn agriculture in north-eastern India I and II, *J Ecol*, **71** (1983) 735-57.
17. Ramakrishnan P S, Let the tribals decide what they want, *Sci-Age*, **2** (1984) 8-11.
18. Ramakrishnan P S, The science behind rotational bush fallow agriculture system (Jhum), *Proc India Acad Sci (Pl Sci)*, **93** (1984) 379-400.
19. Ramakrishnan P S, Tribal man in the humid tropics of the north-east, *Man in India*, **65** (1985) 1-32.
20. Boojh R & Ramakrishnan P S, Growth strategy of trees related to successional status, I and II, *Forest Ecol Mgmt*, (1982) 355-86.
21. Shukla R & Ramakrishnan P S, Architecture and growth strategies of tropical trees in relation to successional status, *J Ecol*, **72** (1986).

## R P Roy

Roy started his teaching and research career in Science College, Patna University, which had provision only for intermediate and BSc pass course till 1948. At that stage, a survey of local flora was initiated, which helped to bring out a monograph entitled "Trees of Patna". This publication continues to date as an important reference material for studies on hardwood and tree plantations in Bihar state. The monograph recorded the presence of a number of important exotic elements like *Adansonia digitata* in the local flora. During this period, a simple leaf-mutant of gram (*Cicer arietinum*) was collected and its basic features were studied and reported.

The studies undertaken on the genome analysis of wheat and its allied genus *Aegilops* at the University of Cambridge were of fundamental importance, paving the way for the future discovery of the source of B genome in the origin of bread wheats. A series of publications based on these studies not only provided valuable insights on the origin and evolution of *Triticum aestivum*, but also prepared the essential groundwork leading to the transfer of the gene for disease resistance from *Aegilops umbellulata* to the cultivated wheats through special cytogenetical techniques.

In the early fifties, plant cytogenetics was almost in its infancy in the country, and through Roy's zeal and untiring efforts after his return from Cambridge, an active centre of research was established at Patna University, which served as a prime mover for many other centres to develop in the country. The first area to attract Roy's attention was the almost barren field of the cytogenetics of Indian hardwoods. He took up detailed cytological studies on the valuable timber-yielding families Dipterocarpaceae (Sal wood family) and Lecythidaceae, and through a number of publications brought out the karyotypic features of species like *Shorea robusta*, *Dipterocarpus alatus*, *Hopea odorata*, *Shorea talura*, *Vatica grandiflora*, *Barringtonia acutangula*, *Careya arborea*, etc.

Almost simultaneously, Roy undertook researches in the cytogenetically neglected groups of plants like the mosses and the ferns. His most noteworthy contributions were on the fern genus *Adiantum* in which hybridization techniques were applied for gaining an in-depth understanding of *A. caudatum* complex. A cytological survey conducted of the ferns of Kathmandu led to the development of a major centre of cytogenetic research on ferns and their allies at Patna University under Roy's pupil, Prof. B M B Sinha.



While basic researches in hardwoods and ferns were being reinforced, Roy applied his attention to the protein-rich group of plants—pulses and other legumes. Valuable contributions emanated on the cytotaxonomy, hybridizations and genetic analyses of various traits of economic importance in a large number of leguminous species.

Through an interesting paper on one of the Irises, Roy was able to bring out the basic features of the nature of chromosome pairing and chiasmata, especially in respect of sister chromatids relation before and after the crossing-over in *Belamcanda chinensis*, which possessed a characteristic heteromorphic bivalent.

Under Roy's guidance, a cytological survey of the grasses of Bihar was undertaken. These studies touched upon the important areas of cytotaxonomy, based, among other chromosomal features, on pachytene analysis. Valuable contributions were made on the environmental control of the mode of reproduction in the facultatively apomictic species, *Dichanthium annulatum*.

Probably the most important area of research to take deep roots under the guidance of Roy is an intensive investigation of the Indian cucurbits, which was commenced in the early sixties and continues to flourish even today. As an initial step, suitable cytological techniques were worked out, which made it possible to obtain excellent chromosomal preparations surmounting the handicaps encountered by earlier cytologists working on cucurbits in India and abroad. During a period of nearly two decades and a half, scores of papers were published by Roy and his colleagues on the cytogenetics of important cucurbit genera like

*Trichosanthes*, *Langenaria*, *Momordica*, *Luffa*, *Cucumis*, *Citrullus*, *Coccinia*, *Edgaria*, *Melothria*, *Gomphogyne*, *Sechium*, *Cyclanthera*, etc. In the course of these investigations, new reports on the chromosome numbers of several species were made, natural polyploids were recorded, intergeneric and interspecific hybrids and amphidiploids were raised and examined cytogenetically, colchipooids were developed, X-irradiation experiments were undertaken and valuable insights were obtained on the mechanisms of sex expression, leading to the establishment of a chromosomal basis of sexual dimorphism in *Coccinia indica*.

Roy's work on the tracing of the evolution of dioecy in cucurbits along three well-marked stages has received wide acclaim. The first step involved the cucurbits like *Luffa echinata*, *Momordica dioica*, *Melothria heterophylla*, where inheritance of dioecism was purely genic in nature and no heteromorphy of chromosomes was involved. The second step was characterized by *Trichosanthes dioica*, in which an 'incipient sex chromosome' appeared to be responsible for maintaining dioecy. This particular pair of chromosomes showed a number of interesting features like (i) heteropycnosis at pachytene, (ii) precocious separation at metaphase I and anaphase I, and (iii) non-disjunction at anaphase II. The third and the ultimate step was demonstrated by *Coccinia indica*, where a distinct X/Y system of sex chromosomes was established with a short X and a long Y with clearly demarcated homologous and differential segments. The contributions on *Coccinia indica* have been referred to in detail in several textbooks on basic genetics and scientific reviews. The main conclusions drawn from the studies on *C.*

*indica* are: (i) karyotypic comparisons established that the male was the heterogametic sex having a chromosomal constitution of  $22A + XY$ , the females having  $22A + XX$ ; (ii) in male meiosis, the sex chromosomes invariably formed a heteromorphic rod bivalent with well demarcated homologous and differential segments; (iii) discovery of an aneuploid with  $22A + XYY$  showed that an occasional non-disjunction of Y chromosome took place at the second meiotic division; (iv) maintenance of strict dioecy in a series of artificially raised polyploids established that polyploidy was not a barrier to dioecy, as was evident in the animals; (v) the Y chromosome was strongly male-determining, as was evidenced by the fact that in triploids and tetraploids, the presence of a single Y chromosome suppressed the feminizing effects of two and even three X chromosomes, giving rise to normal male plants; (vi) genetic evidences were obtained from the recovery of two-subgynoecious plants in the  $R_1$  of X-irradiated diploid *C. indica* to show that mutations on a locus in the X chromosome could lead to the development of a fully fertile male organ in otherwise female flowers, resulting in the appearance of stray perfect flowers on a female plant having a chromosomal constitution of  $22A + XX$ . These conclusions established the justification for using *C. indica* as an ideal specimen for class-room study and research and demonstration of sex mechanism in a dioecious angiosperm. Work on *C. indica*, a perennial climber propagated through seeds and cuttings, has been taken up at several centres to follow up the biochemical pathways in the expression of genes situated on X and Y chromosomes.

The genetic study carried out on the available Indian species of *Luffa* was comprehensive enough to shed valuable light on the evolutionary trends in this genus. Through genetic manipulation, the monoecious *L. cylindrica* (sponge gourd) produced plants which are either male or female. In other words, the derivation of dioecy from bisexual types has been established experimentally; it has also been proved that only two genes are responsible for the expression of sex in these cucurbits. The establishment of a pure female plant in this important vegetable, when fully stabilized, would greatly boost vegetable production.

Although predominantly occupied in cytogenetical investigations in the cucurbits, Roy extended his interest to some other economically important plants and ornaments too. His papers on genera like *Trigonella*, *Indigofera*, *Phaseolus*, *Oryza* and *Bougainvillea* provide valuable commentaries on their cytogenetics.

In the seventies, a well-equipped tissue-culture laboratory was developed under the guidance of Roy, which soon gained eminence through his contributions on anther culture in *Solanum surattense* and other Solanaceous and Cucurbitaceous taxa.

With the acquisition of a fluorescence microscope in the department, Q- and C-banding techniques were extended in three species of *Crinum*, viz., *C. asiaticum*, *C. latifolium* and *C. moorie*. Studies undertaken under Roy's guidance brought out appreciable intraspecific variations in the amount and disposition of heterochromatin/repetitive chromosomal segments. The results obtained were utilized for discussing the role and



phylogenetic significance of heterochromatin in speciation in *Crinum*.

The areas of current research include nucleic acid estimations through Vicker's densitometer-cum-interferometer and visualisations of enzyme polymorphism through gel electrophoresis in a number of cucurbits.

### Selected Publications

1. Roy R P, Semi-lethal hybrids in crosses of species and synthetic amphidiploid of *Triticum* and *Aegilops*, *Indian J Genet*, **15**, 88-98.
2. Roy R P, Genome analysis of *Aegilops sharonensis* Eig, *Genetica*, **29** (1958) 321-50.
3. Roy R P & Saran J, Chiasma frequency in a heteromorphic bivalent in *Belamcanda chinensis*, *Cytologia*, **26** (1960) 253-54.
4. Roy R P & Jha R P, Cytological studies in Dipterocarpaceae. I, *J Indian bot Soc*, **44** (1965) 387-97.
5. Roy R P, Sinha B M B & Sakya A R, Cytology of the fern flora of the Kathmandu Valley, *Br Fern Gaz*, **10** (1969) 193-99.
6. Gupta P K, Roy R P & Singh A P, Aposporous apomixis: Seasonal variation in tetraploid *Dichanthium annulatum* (Forssk) Stapf, *Portug Acta Biol*, **11**(3,4) (1970) 253-60.
7. Roy R P & Roy P M, Mechanism of sex determination in *Coccinia indica*, *J Indian bot Soc*, (Golden Jubilee Volume), **50A** (1971) 391-400.
8. Dutt B & Roy R P, Cytogenetic investigations in Cucurbitaceae. I, Interspecific hybridisation in *Luffa*, *Genetica*, **42** (1971) 139-56.
9. Roy R P, Breeding systems and speciation in higher plants, *Presidential Address, 59th Session Indian Sci Cong* (1972) 1-32.
10. Roy R P, Saran Sunil & Dutt B, Speciation in relation to the breeding system in Cucurbitaceae, *Adv Pl Morph*, (1972) 193-202.
11. Roy R P, Sex mechanisms in higher plants, *J Indian bot Soc*, **53** (3,4) (1974) 141-55.
12. Thakur R & Roy R P, Linkage studies in Indian rice, *Oryza sativa* L, *Euphytica*, **24** (1975) 511-16.
13. Roy R P & Saran J, Mechanism of polyploidisation in endosperm of some angiosperms, *J Cytol Genet (Tandon Memorial Vol)* (9-10) (1974, 1975) 62-72.
14. Zadoo S N, Roy R P & Khoshoo T N, Cytogenetics of cultivated Bougainvilleas. V, Induced tetraploidy and restoration of fertility in sterile cultivars, *Euphytica*, **24**, 517-24.
15. Dutt B & Roy R P, Cytogenetic studies in an experimental amphidiploid, *Caryologia*, **29**(1) (1976) 15-25.
16. Roy R P, Genetic mechanism and significance of outbreeding, *Presidential Address, Nat Acad Sci, India, 46th Annual Session*, 1977, 1-12.
17. Roy R P, Natural and induced haploids and their importance, *J Indian bot Soc*, **57**(4) (1978) 295-305.
18. Sinha Sharda, Roy R P & Jha K K, Segmentation pattern in anther cultures of *Solanum surattense*, *Luffa cylindrica* and *Luffa echinata*, *Phytomorphology*, **28** (1978).
19. Sinha S, Roy R P & Jha K K, Callus formation and shoot bud differentiation in anther culture of *Solanum surattense*, *Can J Bot*, **57**(22) (1979) 2524.
20. Roy R P & Saran S, Sex-expression in the Cucurbitaceae, *Biol & Chem of cucurbits, Symp Vol*, edited by R W Robinson (Academic Press, New York), in press.

## T S Sadasivan

Although the Centre for Advanced Studies in Botany, University of Madras, worked during the first decade on various aspects of 'Soil conditions and soil-borne diseases of crop plants', emphasis during the second and third decades was largely on deranged host physiology under pathogenesis. Nevertheless, the work done in the first phase formed the cornerstone for subsequent experimental investigations in deranged host physiology, and this work alone is presented here for the sake of brevity.

Two model host/pathogen systems were taken up for these long-range experiments because of their relevance to Indian Agriculture; (a) Pathological wilts, with particular reference to the causal agent *Fusarium vasinfectum* on cotton and *Fusarium udum* on pigeonpea (*Cajanus cajan*); and (b) Blast disease of rice caused by *Pyricularia oryzae*.

### (a) Pathological Wilts

(i) *Pectin in wilted plants*: Comparison of the cotton variety susceptible to the wilt organism *F. vasinfectum*, with the variety resistant to it indicated rapid depletion of pectin reserves in the former. As pectic enzymes play a major role in the formation of tyloses due to hyperauxiny, our results partly explained the wilt syndrome. In fact, interest in discovering fungal strains with little or no fungal enzyme producing ability

stemmed from some of this basic work here and elsewhere.

(ii) *Carbohydrate substrate*: Our group turned its attention to a detailed study of carbohydrates in infected roots of wilted plants. Decrease in reducing sugars and in total carbohydrates was recorded in roots of susceptible pigeonpea plants infected with *F. udum*, whereas in a less susceptible variety, the reducing sugar level was little affected.

(iii) *Respiratory changes*: Studies in respiratory changes revealed that there is a progressive increase in respiratory rates in the root tissues of susceptible cotton infected with *F. vasinfectum* as compared with the resistant variety; the increase was initially high, but declined with age. Our group also showed that toxins produced by several fusaria stimulated respiration of plant tissues. This was a period when intensive work in other laboratories on the effect of many fungal toxins indicated profound effects on respiration of host tissues. Our group subscribed to the view that stimulation of respiration by toxins of the pathogen could be the result of uncoupling of phosphorylation from electron transfer. Further, several enzyme systems seemed to be activated in the infected tissues, yet not all of these systems were shown, beyond doubt, to be involved in electron transport *in vivo*.



(iv) *Nitrogenous compounds*: Roots of susceptible intact plants infected by the fungus *F. vasinfectum* showed a decrease in total nitrogen levels, while resistant plants showed an accumulation. An increase in protein nitrogen occurred in both susceptible and resistant plants grown in infected soils. However, non-protein nitrogen decreased on infection in susceptible roots, whereas it increased in the resistant varieties.

(v) *Water relations*: The uptake of water and the ionic status of the intact wilting plants engaged our attention for many years. As against the use of shortcuts for such assays, our group used infected plants grown under normal soil conditions. An increased conductivity was recorded in *F. vasinfectum* infected cotton roots; this was ascribed to accumulation of electrolytes due to primary changes in osmoregulation of the host cells. In the susceptible variety of cotton, the iron content in inoculated plants was lower than that in healthy plants in the early stages of infection, but at the peak level of wilting, there was an accumulation of iron. As against this, in the resistant variety grown in infected soil, the plants had more iron compared to the control during the early stages of incipient infection and much less so during the later stages of growth. In the case of infected pigeonpea plants, Fe/Mn ratio in the wilting plants was directly proportional to the degree of susceptibility of the cultivars. An exaggerated transpiration during the shock phase has been established by our group in the two-model host-pathogen systems used in our studies. Following this shock phase, there was a gradual fall in the transpiratory rate in the diseased plant tissues as compared to the healthy plant

tissues. Much of our work was undertaken with fusaric acid, a non-specific toxin of low molecular weight and produced by many fusaria. Somewhat similar investigations were conducted elsewhere also, using the tissue specific toxin victorin (from the pathogenic fungus *Helminthosporium victoriae*) which reduced transpiration not only of the specific susceptible victoria variety of oats, but also of the resistant variety.

Quite naturally, we argued that loss in turgor brought about by a fluctuating abnormal to sub-normal water transport brings in its trail ionic imbalance. The vivotoxin fusaric acid produced by the fungus *F. vasinfectum* has been identified by us *in situ* from infected soils and in the rhizosphere of cotton plants. It is stable in the rhizosphere of crop plants and is known to impair membrane permeability at fairly low concentrations. A chain of primary changes in osmoregulation of host cells following toxemia is followed by increased conductivity and accumulation of electrolytes, resulting in increased ionic imbalance. However, the most surprising find by the Madras group was a very heavy foliar loss of the key metabolite, potassium. Calcium, magnesium and manganese levels increased in the tissues. The result obtained here with the non-specific vivotoxin fusaric acid was confirmed elsewhere using some of the specific pathotoxins like victorin, where leachates from victorin-treated susceptible tissues contained accumulated nitrogen, amino acids, carbohydrates and inorganic phosphorus as compared to the untreated tissues. In addition, as in our experiments with fusaric acid and the cotton tissue, there was a four-fold loss of potassium, resulting in loss in turgidity of leaves and, in its trail, ionic imbalance with

progression of the wilt syndrome. Therefore, the physiological changes in pathological wilting are many and complex. It seems difficult to draw a line between induced changes and degradative changes of a crippled metabolism. In essence, we realized and put forward the view that wilt induction was intimately connected with loss in turgor, deranged osmoregulation of cell wall function, changes in the plasmalemma, the protoplast and the tonoplast. In the case of the tissue specific toxin victorin, treatment enhanced the synthesis of malic and citric acids, but had no effect on aconitic and succinic acids. The enhanced synthesis of malic acid was ascribed to a release from the mitochondria which acted as a buffering agent for excess cations that leak from the vacuole. Many of the fundamental points raised by us on changes in osmoregulation leading to changes in plasmalemma have been confirmed in the case of victorin at sub-cellular level at other centres in this field of research.

## (b) Rice Blast

(i) *Environment conditioning of host susceptibility* : The complex nature of rice blast was investigated, in depth, in the third phase of our research programme. The main thrust in the area of changes in phenolics and carbohydrates and correlating this with night temperature and pathogenicity was undertaken in rice plants (susceptible, tolerant and resistant varieties) infected with *Pyricularia oryzae*. The main point was that resistant and susceptible phenotypes are not infected when these rice plants are grown in controlled night temperatures above 26°C, but succumb to infection when they are grown at a night temperature of 20°C. An

incompatible genotype nycto-temperature interaction seemed to endow the resistant variety with a firmer cuticle. This may result in a retarded nitrogen metabolism and as a consequence in the shunting off of the carbon skeletons to structural metabolism of cuticle and wax synthesis.

(ii) *Host-nitrogen status and disease proneness* : On the contrary, a compatible genotype-night temperature interaction in the susceptible variety may stimulate nitrogen metabolism and lessen the chances of an optimum structural metabolic function that builds up the outermost defence barrier, such as the cuticle. Our group worked out in great detail changes in host nitrogen metabolism and phenolics of susceptible and resistant rice plants. The net gain in protein in the susceptible variety early in infection indicated an increase in protein synthesis following infection. Subsequently, such increases were not recorded, indicating a block in protein synthesis with advancing pathogenesis. This was borne out by the fact that much accumulation of glutamine took place in the susceptible plant early in infection, showing a higher protein metabolism compared to the healthy controls. The main interest lay in understanding the effect of night temperature and host-parasite relations in blast disease. Among the many leads we obtained, the most important ones are mentioned below.

(iii) *Nitrogen metabolism*: The augmented nitrogen metabolism may not be the sole determining factor in susceptibility. Nevertheless, accumulation of nitrate in rice leaves grown at high night temperatures pointed to the failure of nitrate reductase activity and this brought in its trail shunting of the carbon



compounds to structural materials like cutin and waxes. Indeed, the nitrate reductase system of the susceptible rice variety, when grown at 20°C nycto-temperature, gets stimulated, resulting in the incorporation of nitrogen in the amide. However, such a stimulation was not noticed in the resistant variety where glutamin resistance factors seemed to operate through the nitrate reductase system, which, in turn, is dependent on genetic inheritance.

(iv) *Nycto-temperature and phenotypic response of host to infection*: A new concept of phenotype/genotype/biotype interaction emerged from many of these experiments. We had indicated earlier increased susceptibility to the blast disease of a susceptible genotype, where the exposure of the susceptible rice plants to low night temperature regimen of 20°C for a period of 10-15 days increases its susceptibility and proneness to blast. Subsequently, two biotypes of *Pyricularia*, one operating at 20°C nycto-temperature and the other at 15°C brought into play three factors instead of two. We, therefore, re-defined the disease syndrome as a phenotypic expression of a genotype in relation to the biotype of the pathogen. The whole problem is complex, as *Pyricularia* isolates, whether they fall into the 15°C or 20°C biotype categories, surprisingly came from the alternative grass hosts of the fungus. For these

grasses—*Panicum repens*, *Leersia hexandra* and *Brachiaria mutica*—and their role in the triple interaction, it is taken for granted that we are dealing with single genomes among the grass populations. It may well be that these common grasses could have much variability in a given population, as between the extreme cases of rice differentials used in our experiments.

### Selected Publications

1. Sadasivan T S, Physiology of wilt disease, *A Rev Pl Physiol*, **12** (1961) 449-68.
2. Sadasivan T S, Respiration in plants under pathogenesis, *Indian Phytopath*, **17** (1964) 263-72.
3. Sadasivan T S, Physiology of wilting plants, in *Biochemische Probleme der kranker Pflanze, Tagungsberichte No 74 (DDR)* (1965) 149-63.
4. Sadasivan T S, Nitrogen metabolism and resistance to facultative parasites, in *Biochemical regulation in diseased plants or injury* (Phytopathological Society of Japan, Tokyo), 1968, 239-52.
5. Sadasivan T S, Physiology and plant pathology, *Proc Indian Acad Sci*, **59 B** (1969) 95-103.
6. Sadasivan T S, On better understanding of plant pathogens, *Proc Indian Acad Sci*, **74B** (1971) 113-36.
7. Sadasivan T S, Physiological plant pathology: A multidisciplinary science, *Proc Indian natn Sci Acad*, **40B** (1974) 347-58.
8. Sadasivan T S & Kalyanasundaram R, Phytotoxins and toxemia, *Beitrage zur Biochemie und Physiologie von naturstoffen* (Festschrift Kurt Mothes, Geburtstag, Veb Gustav Fischer Verlag, Jena) 1965, 379-83.
9. Sadasivan T S, Subramanian C V, Kalyanasundaram R & Saraswathi Devi L, Pathological wilting of plants, *Proc Symp Univ Madras*, 1978, 527.

## S B Saksena

Saksena's researches fall under three broad heads: (i) Ecological studies; (ii) Fungal systematics; and (iii) Pathological aspects.

### Ecological Studies

Edaphic factors are known to govern the distribution of surface vegetation on land to a large extent. The vegetational types can often be characterized in correlation with the soil types. A vital point is whether the microbiological flora also varies with the soil type and could be similarly characterized. Studies in this connection were carried out in 'Patharia' forest near Sagar (Madhya Pradesh) which has several types of soils with characteristic surface forest flora. Sites representing five different soil types were selected for this study; in addition, one grassland type and one forest type (situated far away) were chosen as contrasting types. The surface vegetation was analysed phytosociologically for frequency, abundance and dominance of each tree species.

Phytosociological analyses of data on fungal flora revealed that pioneer species like *Penicillium funiculosum* and *Aspergillus fumigatus* were confined to poor conditions of soil and could stand the trying hot condition. These may serve as useful indicators of such poor soils. On the other hand, a number of species appear

only in the climax conditions and do not appear in earlier successional stages. These are: *Gliocladium deliquescens*, *G. roseum*, *Aspergillus variegator*, *Cunninghamella bertholletiae* and *C. verticillata*. These would be good indicators of mature and well developed soils. Finally, there were the ubiquitous species, *Aspergillus niger*, *Absidia spinosa* and *Penicillium nigricans*, which appeared to have a high ecological tolerance. Though these could survive any conditions, their crest of frequency was reached in good and mature soils.

The number of species and the total number of fungi were found to increase progressively from the pioneer to the climax type of soils and it was revealing to find a striking parallelism with the higher vegetation in the successional sequence from the pioneer to the climax types.

### Fungal Systematics

During these studies, several new and important genera and species were discovered. The notable among these are: *Saksenaea vasiformis* gen et sp nov; *Monocillium indicum* gen et sp nov; *Gliocladiopsis sagariensis* gen et sp nov; *Cephalosporium roseo-griseum* sp nov; *Sporothrix albicans* sp nov; and *Paecilomyces fusisporus* sp nov. Among these, *Saksenaea vasiformis* proved to be

---

Formerly, Professor & Head, Department of Botany, Saugar University, Sagar, MP; Residence : 55 Shri Ram Colony, Jhansi Road, Gwalior-434002.



of great pathological value. It is also of great significance in fungal systematics and a new family Saksenaeaceae had to be created to accommodate it. It has a peculiar structure of the sporangium—a beautifully perfected flask with a long neck and globular venter and a very well developed columella. By the time it reaches maturity, it develops a well developed mucilaginous plug, which fits snugly in the mouth of the flask. A large number of spores are oval-oblong in shape. At the base a radially symmetrical rhizoidal structure develops which consists of dichotomously branched stout hyphae. This lower portion is pigmented brownish. At maturity, the mucilaginous plug dissolves away to open the mouth of the sporangium through which the spores are liberated.

The well developed coenocytic hyphae with chitinous walls, the non-motile spores produced in abundance and the well developed columella led us easily to place the fungus undoubtedly in mucorales. On the other hand, the shape and structure of the sporangia, the long neck with a mucilaginous plug, the mode of liberation of spores do not permit us to place it in any of the existing families.

The peculiar structures met with in this genus throw some fresh light on the possible ancestry of mucorales, a much debated question. There are two main lines of thought. According to one school, the group has originated from some aplanosporic saprolegniaceous forms which inhabited soil. The other school believes that the mucorales have come down from some Chytridiaceous ancestors. The discovery of *Saksenaea vasiformis* lends support to the latter hypothesis because of the flask shaped

sporangium with long necks, guided opening with mucilaginous plug and chitinous cell walls. Except for the aplanospores (which would replace zoospores), the entire structure would resemble those of some Chytridiaceous forms.

### Pathological Aspects

(i) *Saksenaea vasiformis* : The fungus *S. vasiformis* has been established as a virulent human pathogen which causes zygomycosis in human tissues. Zygomycosis which causes tissue infections in human-beings has been known for a long time. The causal organism could not be specifically identified, as the fungus did not produce any reproductive structures in cultures. The presence of unseptate rapidly growing hyphae with chitinous walls was thought to be sufficient to place the organisms in Zygomycetes. The infections became extremely serious and proved fatal in some cases in spite of amputations of the affected parts.

Recently, some of the isolates of zygomycosis have been cultured on hay infusion agar and Czapek's agar in which they sporulated to produce typical sporangia of *Saksenaea vasiformis*, thus establishing the specific diagnosis of zygomycosis.

(ii) *Ecology of Trichoderma viride* . Studies in soil microbiology have revealed that some antagonistic organisms in soil are responsible for controlling root disease organisms in it. One such known antagonist is *Trichoderma viride* which is widespread in soils. It is also known that the population of *T. viride* increases rapidly in fumigated and steam sterilized soil. This preponderance is usually

explained on the basis of its tolerance to the fumigant. This particular aspect was studied by Saksena at Cambridge University. The effect of carbon disulphide fumigation on the soil fungus flora was studied over the range 0.05-20.0 ml carbon disulphide/250 g soil in Evan's recolonization tubes.

The experiments conducted permitted the conclusion that the dominance of *T. viride* in soil fumigated with carbon disulphide can be attributed to a moderate degree of tolerance to the fumigant allied with an intrinsically high growth rate. This enables the fungus to colonize volumes of soil sterilized by the fumigant in advance of most of its fungal competitors.

(iii) *Foot rot and leaf rot of Pan (Piper betle)* : Foot rot and leaf rot of Pan is rampant in parts of Madhya Pradesh and other parts of India, where betel vine is cultivated. This deadly disease is caused by *Phytophthora parasitica* and is responsible for enormous losses annually. Some ecological aspects of this pathogen were studied by Saksena and some of his associates at the Saugar University. They found that when cuttings of the plant are dipped in streptomycin solution and spray of 1% Bordeaux mixture is given twice a

month, the disease is controlled almost totally.

### Selected Publications

1. Saksena S B, A new genus of mucorales, *Mycologia*, **45** (1953) 426-36.
2. Saksena S B, A new species of *Paecilomyces* from soil, *J Indian bot Soc*, **32** (1953) 186-89.
3. Saksena S B, A new genus of moniliaceae, *Mycologia*, **46** (1954) 660-66.
4. Saksena S B, A new fungus *Monocillium indicum* Gen et sp nov from soil, *Indian Phytopath*, **8** (1955) 9-12.
5. Saksena S B, A new species of *Cephalosporium*, *Mycologia*, **47** (1955) 895-98.
6. Saksena S B, Ecological factors governing the distribution of soil microfungi in some forest soils of Sagar, *J Indian bot Soc*, **34** (1955) 262-98.
7. Saksena S B, Effect of carbon disulphide fumigation on *Trichoderma viride* and other soil fungi, *Trans Br mycol Soc*, **43** (1960) 111-16.
8. Saksena S B, A new species of *Sporothrix*, *Curr Sci*, **34** (1965) 318.
9. Saksena S B, Root diseases and biological control (Presidential Address), *J Indian bot Soc*, **51** (1972) 1-13.
10. Saksena S B, *Phytophthora parasitica*, the scourge of pan (*Piper betel*), *Indian Phytopath*, **30** (1977) 1-16.
11. Saksena S B, The genus *Phytophthora* in India, *Kavak*, **7** (1979) 1-16.
12. Saksena S B, On the affinities and biology of *Saksenaia vasiformis*—An unusual mucoraceous fungus (Presidential Address), *Proc Indian Sci Cong*, **66** (Pt II) (1979) 1-16.



## Y S R K Sarma

My researches spanning over almost a quarter of a century have been devoted mainly to the study of algae, with particular reference to their karyology. The highlights of my contributions are presented below in brief.

### (1) Cytology and Cytotaxonomy of Several Groups of Algae

The work in this area covers more than 300 taxa of algae, largely belonging to green algae, and some to Dinophyceae. The prominent karyological characters of the orders Volvocales, Ulotrichales, Chaetophorales, Oedogoniales, Conjugales, Siphonales, included under Chlorophyceae, Charophyceae, Bacillariophyceae, Euglenophyceae and Dinophyceae, have been summarized in several reviews<sup>1-5</sup>. Among the algal taxa whose chromosome numbers were recorded from our school, more than 200 are first reports. Ours is the only centre in India which has worked out the karyology of Indian freshwater Dinophyceae, a highly interesting group of algae from the viewpoint of cytology.

On the basis of chromosome number, breadth and length of individual chromosomes, N O chromosomes, if detectable, it has been possible to prepare idiograms of individual taxa belonging to Charales<sup>6</sup> and Oedogoniales<sup>7</sup>, which proved of immense value in discussing the

evolutionary trends within these groups. Although a large number of taxa of Cladophorales were investigated by other workers, no concerted effort had been made to depict idiograms of karyotypes, although the chromosomes are appreciably large and centromeric position of individual chromosomes is identifiable.

Karyological studies were made use of by Sarma and colleagues to determine the systematic position of certain genera of controversial position. For example, *Sphaeroplea* has been shown to be more closely related to Ulotrichales than to Cladophorales or Siphonales on the basis of its karyology<sup>8</sup>. Similarly, *Microspora* was shown to be having close affinities with Ulotrichales on cytological ground<sup>9</sup>. Many more instances have been quoted by Sarma<sup>3</sup>.

On the basis of cytographic and cytosystematic survey of world literature on Charophyta, Khan and Sarma<sup>10</sup> have put forth the view that possibly India (particularly Uttar Pradesh and Bihar) may be considered as the primary centre of origin for world charophyte flora.

### (2) Cytogenetics of Algae

From a review of the work on the effect of irradiation on green algae, Sarma<sup>11</sup> concluded that most of the green algae are highly resistant to ionizing radiations (in comparison to higher plants), members of

---

Formerly, Professor of Botany, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005; Residence : B7/12, Sheesh Mahal Colony, Kanachha, Varanasi-221010.

Conjugales, Chlorococcales and Euglenoids being notable in this respect. Those algae that are characterized by appreciably long chromosomes with localized centromeres, such as *Oedogonium* spp., and the members of Charales seem to be comparatively more sensitive.

Sarma and Agarwal<sup>12</sup> studied for the first time the effects of ultrasonic waves on the karyology of two green algae, *Rhizoclonium heiroglyphicum* and *Oedogonium gunnii*. The karyological effects were found to be akin to those obtained with radiations. Sarma<sup>13</sup> reviewed the work on the karyological effects of antibiotics (chloramphenicol, oxytetracycline, gentamycin, pencillin, streptomycin, mytomyacin-C, rifamycin and polymixin-B) on green algae, *Oedogonium gunnii*, *Spirogyra azygospora* and *S. paradoxa*. Gentamycin and polymixin-B were found to be more potent with respect to lethality as well as in the extent of production of comparable nuclear and chromosomal aberrations.

Our school has studied the karyological effects of several chemicals, besides antibiotics, on some members of green algae (Sarma and Abhyavardhani, in press). It has been concluded that, with a few exceptions, green algae are more resistant to chemicals as compared to higher plants. Several interesting morphological and cytological abnormalities were recorded in the studies on the effects of NTG on *Cosmarium obtusatum*. Of particular interest is the induction of giant cells with doubled chromosome number, besides the formation of variously shaped longer cells than the control organisms, formation of chains of incompletely separated cells and

occasional formation of chains of incompletely separated cells and occasional formation of triradiate cells.

Our school has carried out extensive studies on the modifying action of certain chemicals on the effects of ionizing and non-ionizing radiations in various selected experimental green algae. It was shown that all the chemicals tested provided radio-protection to the algae when employed in pretreatments. In post-treatments, however, some chemicals proved to be synergistic to radiations, while others were antagonistic. All the work carried out by our group has been reviewed recently by Sarma and Agrawal<sup>12a</sup>.

### (3) Physiological Studies

The effects of UV light, gamma radiation and other factors, such as quality of light, intensity of white light, temperature, pH and nutrients on the various phases of the life cycle in the alga were studied<sup>14-16</sup>. The mode of germination and effects of UV light on germination and the extent of recovery of UV effects through photoreactivation were studied in respect of akinetes of *Pithofora*<sup>17</sup>. Notable success was achieved in the formation of new synthetic nitrogen depleted medium in which conjugation was induced in two desired taxa, *Closterium acerosum* and *C. turgidum*. A new synthetic culture medium was devised supporting better growth of several freshwater dinoflagellates.

### (4) Systematics, Morphology and Reproduction

While our studies are concentrated mainly on the karyology of algae, a few notable contributions have emerged on the systematics, morphology and reproduction of some algae. From systematic studies on



North Indian Volvocales, a significant discovery has been that of a new colonial genus, *Lundiella indica* gen et sp nov, in which the coenobia are obovoid in shape with slightly flattened apical region. The cells of the colony are polyhedral in shape, arranged on the periphery without any sequence of tiers, and joined to one another by mucilagenous pads between laterally produced portions of the cells, but without a common mucilagenous envelope. This genus is at present known only in its vegetative state<sup>18</sup>.

Eight unicellular taxa of Volvocales, belonging to the genera *Papenfussiomonas*, *Chlamydomonas*, *Haematococcus*, *Sphaerellopsis* and *Phacotus* have been described by Shyam and Sarma<sup>19</sup>, of which two new varieties, *S. fluviatilis* var. *striata* var. nov. and *P. lenticularis* var. *undulata* var. nov. were established. Two new species of *Pyrobotrys* (*P. acuminate* sp. nov. and *P. desikacharyi* sp. nov.) have also been described by Sarma and Shyam<sup>20</sup>. Some new species were established by the same workers in Indian Dinophyceae<sup>20a</sup>.

Some new facts concerning the sexual reproduction in *Eudorina californica* (Shaw) Goldstein were brought to light by Shyam and Sarma<sup>21</sup>, with reference to the place of actual union of gametes, details of actual fusion and time taken to complete the process. Certain new observations on the formation, liberation and fusion of biciliate gametes have been made in the alga *Tetrasporidium javanicum* by Sarma and Suryanarayana<sup>22</sup>.

With the sole objective of providing information about the scattered literature on algal taxonomic work in India at one place, to beginners in algal systematics, a check list of algal taxa published from

India up to 1976 along with a list of source references was prepared and released by Sarma and Khan<sup>23</sup>.

### Selected Publications

1. Sarma Y S R K, Algal caryology in India, *Advancing Frontiers in Cytogenetics* (1973) 266-85
2. Sarma Y S R K, Chromosomes of algae, *Proc UGC Symp Recent Trends and Contacts between Cytogenetics, Embryology and Morphology*, 1977, 155-65.
3. Sarma Y S R K, Cytotaxonomy in algae, *Glimpses in Pl Res*, **5** (1979) 20-44.
4. Sarma Y S R K, Chromosome numbers in algae, *Nucleus*, **25** (1982) 66-108.
5. Sarma Y S R K, Algal caryology and evolutionary trends, in *Chromosomes in evolution of Eukaryotic groups*, Vol 1, edited by A K Sharma and A Sharma (CRC Press, Florida) 1983, 178-223.
6. Khan M & Sarma Y S R K, Studies on the cytotaxonomy of Indian Chrophyta. I. *Chara*, *Phykos*, **6** (1967) 36-47; II. *Nitelb*, *Phykos*, **6** (1967) 48-61. II. *Nitella*, *Phycos*, **6** (1967) 48-61.
7. Srivastava S & Sarma Y S R K, Karyological studies on the genus *Oedogonium* Link (Oedogoniales, Chlorophyceae), *Phycologia*, **18** (1979) 228-36.
8. Sarma Y S R K, Nuclear cytology of *Sphaeroplea annulina* (Roth) Ag and its bearing on the systematic position of *Sphaeroplea*, *Cytologia*, **27** (1962) 72-78.
9. Sarma Y S R K, Contributions to the karyology of the Ulotrichales. III, *Microspora* Thuret, *Nucleus*, **6** (1963) 49-62.
10. Khan M & Sarma Y S R K, Cytography and cytosystematics of Charophyta, in *Systematics of green algae*, edited by DEJ Irvine & DM John, *Systematics Association, Special Volume No 27* (Academic Press, London, Orlando) 303-30.
11. Sarma Y S R K, Irradiation studies on green algae—A résumé in *Recent advances in cryptogenic botany*, edited by D C Bhardwaj (Paleobotanical Society, Lucknow) 1981-82, 16-32.
12. Sarma Y S R K & Agrawal S B, Effects of ultrasonic waves on the karyology of two green algae, *Nucleus*, **24** (1981) 75-79.
- 12a. Sarma Y S R K & Agrawal S B, Modifying action of certain chemicals in changing the effects of

- radiations in green algae, in *Trends in plant research*, edited by C M Govil & V Kumar (Bishan Singh, Mahendra Pal Singh, Dehradun) 1985, 12-18.
13. Sarma Y S R K, Karyological effects of antibiotics on algae, in *Contemporary trends in plant sciences*, edited by S C Verma (Kalyani Publishers, Ludhiana) 1982, 88-92.
14. Sarma Y S R K & Agrawal S C, Effects of UV light on sporulation of the green alga *Stigeoclonium pascheri* (Vischer) Cox & Bold, *Indian J exp Biol*, **18** (1980) 298-300.
15. Agrawal S C & Sarma Y S R K, Effects of nutrients present in Bold's basal medium on the green alga *Stigeoclonium pascheri*, *Folia Microbiol*, **27** (1982) 131-37.
16. Agrawal S B & Sarma Y S R K, Effects of some physical factors and pH on the sporulation of green algae *Stigeoclonium pascheri* (Uischer) Cox & Bold, *Indian J Bot*, **5** (1982) 151-54.
17. Sarma Y S R K, Chaudhary B R & Agrawal S B, UV light and photoreactivation studies on the germination of akinetes of *Pithophora kewensis*, *Bibi Phycol*, **66** (1983) 321-31.
18. Sarma Y S R K & Shyam R, *Lundiella indica* gen et sp nov, a new member of colonial Volvocales (Chlorophyceae) from India, *Br phycol J*, **9** (1974) 307-11.
19. Shyam R & Sarma Y S R K, Studies on the North Indian Volvocales. IV. Some interesting unicellular forms, *Arch Protistenk*, **118** (1976) 107-18.
20. Sarma Y S R K & Shyam R, Studies on North Indian Volvocales. I. The genus *Pyrobotrys Arnoldi*, in *Taxonomy of algae (Proc Int Symp)*, edited by T V Desikachary & V N Raja Rao (University of Madras) 1980, 321-35.
- 20a. Sarma Y S R K & Shyam R, On the morphology, reproduction and cytology of two new freshwater dinoflagellates from India, *Br phycol J* **9** (1974) 21-29.
21. Shyam R & Sarma Y S R K, Studies on the North Indian Volvocales. III. Fertilization in *Eudorina californica* (Shaw) Goldstein, *Hydrobiologia*, **50** (1976) 113-16.
22. Sarma Y S R K & Suryanarayana G, Observations on the morphology, reproduction and cytology of *Tetrasporidium javanicum* Moebius from North India, *Phycologia*, **8** (1969) 171-75.
23. Sarma Y S R K & Khan M, *Algal taxonomy in India—Botanical Records and Monographs—2* (Today & Tomorrow's Printers & Publishers, New Delhi) 1980.



## S P Sen

I still remember that day in the summer of 1947 when Prof S M Sircar agreed to accept me as an MSc thesis student. It was a thrill. A few years earlier, Prof. Sircar had shown for the first time that *Aman* (winter) varieties of rice could be forced to flower in summer by exposing them to short day lengths. He wanted me to follow the changes that take place in the shoot apices of a winter variety of rice, *Rupsail*, when it is subjected to photo-inductive cycles. I was fortunate in having a glimpse of a great mind, which had influenced considerably my scientific approaches. His laboratory on the ground floor of 35, Ballygunge Circular Road, Calcutta, had very few facilities, but I enjoyed thoroughly the long hours I used to spend there. A few years later, the results were published in *Nature*<sup>1</sup>. It was also here that I predicted on theoretical considerations, later substantiated by experimental evidence, that the *Aman* varieties of rice would also flower in spring—an observation which formed the basis of growing *Aman* rice crop twice a year<sup>2</sup>. That laboratory was housed in an old dilapidated building, which has now been demolished to make way for the seven storeyed building which now houses the Department of Botany, Calcutta University.

Two years later, in the early summer of 1949, I approached with trepidation Dr D

M Bose, the then Director of the Bose Institute, with the request for a research scholarship, which was bluntly turned down. A few days later, I was surprised to receive an appointment letter to work with Prof. P N Nandi, who had returned a few months earlier after a fruitful stay at the Imperial College of Science & Technology, London. Those were the early days of antibiotics; pencillin and streptomycin were just beginning to be sold on a commercial scale. I worked on the isolation of antibiotic-producing soil microorganisms and studied their effects on N<sub>2</sub>-fixing bacteria. It was during this period that I first showed the occurrence of antibiotic properties in extracts of pteridophytes<sup>3</sup>, which were later studied in detail. It was also during this period that I collaborated with Dr D P Burma on paper chromatographic analysis of plant extracts, particularly the amino acids of root nodules.

In November 1952, I was offered a fellowship at Purdue University to work with Prof. A C Leopold, another great plant physiologist, who has influenced considerably my scientific thoughts. John E Christian trained me up in isotope methodology, which I used in my researches on the physiology of flowering and showed the influence of light and darkness on CO<sub>2</sub> fixation in photoperiodism<sup>4</sup>. Work on paper chromatographic

---

Professor, Botany Department, Kalyani University, Kalyani-741235; Residence : P 10/251, Kalyani University Campus, Kalyani-741235.

separation of natural and synthetic auxins was also done during this period and the use of water as a developing solvent for the separation of neutral auxins was described<sup>5</sup>.

Summoned by Dr D M Bose, I returned to India to set up the Radiochemical Laboratory of the Bose Institute in 1954. In the late fifties, together with B B Biswas, I studied the path of sulphate and phosphate during the early period of uptake, the reduction of sulphate, the role of thioctic acid in photosynthesis and plant hormone effects on the synthesis of DNA and RNA. A Sen Gupta, who had joined my laboratory by this time, showed auxin effects of CO<sub>2</sub> fixation<sup>6</sup> and metabolic processes and the need of reduced pyridine nucleotides in auxin-induced growth. R Roychoudhury and Anima Datta developed this work further and showed almost simultaneously with K V Thimann at Harvard, the importance of RNA synthesis in hormone-induced growth<sup>7,8</sup>.

In 1961, I joined Kalyani University to establish the Department of Botany there and since then my research group has included both plant physiologists and microbiologists. R Mitra and J Das studied in detail the mechanism of hormone action, particularly in relation to interaction with macromolecules and membrane components. J Guha discovered the antigibberellin activity of the tetracyclic, triterpenoid cucurbitacins<sup>9</sup> and later S Mondal and U Das showed that this property is exhibited by the pentacyclic triterpenoids as well. S N Seal and C Choudhuri showed the importance of nucleic acid and protein synthesis in the ageing of plant and animal tissues. B R Mitra and D N Sengupta carried out extensive studies on the role of RNA and protein synthesis in photoperiodic

induction of flowering, both at cellular and subcellular levels<sup>10</sup>. It was shown that in day-neutral plants, the same events take place as a function of time what photoperiods achieve in photosensitive plants. The control of differentiation and morphogenesis at the molecular level was demonstrated by J K Ghosh, A Jana and R Maitra. The molecular basis of mineral deficiency effects was worked out by B K Das, who showed that RNA synthesis is interfered with very early under deficiency condition before other metabolic processes are influenced<sup>11</sup>. D N Sengupta and N Maitra made critical studies on source-sink relationships, particularly in relation to the presence of inhibitors of photosynthesis in the sink organs and the control of metabolite mobilization from source organ by regulators produced in the sink organ.

Genetic transformation in *Azotobacter* was first demonstrated by M Sen<sup>12</sup>; the transference of N<sub>2</sub>-fixing properties to non-N<sub>2</sub>-fixing strains was also first shown in 1967<sup>13</sup>. Genetic transformation in pseudomonads, yeasts, fusaria and several N<sub>2</sub>-fixing and antibiotic producing micro-organisms was described by N C Khan, T K Paul and G D Biswas. An observation of considerable importance was recorded by B Sengupta, A S Nandi, D Paul, R Samanta, A Nandi and others<sup>14</sup> that N<sub>2</sub>-fixing micro-organisms are widespread in the leaves of tropical plants and the more active strains when sprayed on crop plants meet to a large extent the nitrogen requirement of such plants. The utility of *Rhizobium* in ectosymbiotic association was also demonstrated. R K Misra showed the occurrence of N<sub>2</sub>-fixing bacteria in nodule-like structures of several non-leguminous dicotyledonous herbs. Another observation of far-reaching



significance was the demonstration by R K Samanta that the yeast *Lipomyces starkeyi* and many cucurbits<sup>14</sup> are capable of fixing N<sub>2</sub> under aseptic conditions. These are the first reports of N<sub>2</sub> fixation by eukaryotes.

A K Sadhukhan, D G Datta Roy and M K Pasha carried out extensive studies on DNA homology of a large number of plant taxa. The observations suggest that many plant taxa are polyphyletic in origin and several current theories of evolution are in need of revision<sup>15</sup>. The extent of occurrence of repeated sequences in the different groups of plant kingdom has also been studied.

My long association with the Bose Institute, well known for its researches on the living and non-living under the leadership of J C Bose and D M Bose, had nurtured my interest in the origin and evolution of life. The thoughts were collected in my Presidential Address to the Botany Section of 72nd Indian Science Congress held at Lucknow in 1985<sup>16</sup>.

### Selected Publications

1. Sircar S M & Sen S P, Photoperiodic induction and the development of growing apex in rice, *Nature, Lond*, **165** (1950) 855; also *Bot Gaz*, **114** (1953) 436-48.
2. Sircar S M & Sen S P, Interaction of temperature and day length in flowering of winter paddy, *Rupsail, Curr Sci*, **29** (1951) 238-39.
3. Sen S P & Nandi P N, Antibiotics from pteridophytes, *Sci & Cult*, **16** (1950) 328; also *Econ Bot*, **34** (1980) 284-95.
4. Sen S P & Leopold A C, Influence of light and darkness upon CO<sub>2</sub> fixation, *Pl Physiol*, **31** (1959) 323-29; also *Indian J Pl Physiol*, **5** (1962) 202-17.
5. Sen S P & Leopold A C, Paper chromatography of plant growth regulators and allied compounds, *Physiol Plant*, **7** (1954) 98.
6. Sengupta A & Sen S P, Carbon dioxide fixation by auxin treated tissues, *Pl Physiol*, **36** (1961) 374-80.
7. Roychoudhury R & Sen S P, Metabolic conversion of thymine 2-C<sup>14</sup> and its incorporation into nuclear RNA of endosperm nuclei of *Cocos nucifera* Linn, *Biochem Biophys Res Commun*, **14** (1964) 7-11.
8. Sen S P & Das J L, Probable sites of plant hormone action, in *Recent Developments in Plant Sciences*, edited by S P Sen (Today and Tomorrows Printers and Publishers, New Delhi) 1981, 61-78.
9. Guha J & Sen S P, Anti-gibberellins from the Cucurbitaceae, *Nature (New Biol), Lond*, **244** (1973) 223-24.
10. Sen S P, Some biochemical aspects of the initiation of reproductive phase in plants, *Indian Rev Life Sci*, **2** (1982) 67-95.
11. Das B K & Sen S P, The nature of N,P,K-deficiency effects in rice, *Pl Cell Physiol*, **21** (1980) 1243-54.
12. Sen M & Sen S P, Interspecific transformation in *Azotobacter*, *J gen Microbiol*, **41** (1965) 1-6.
13. Sen M, Pal T K & Sen S P, Intergeneric transformation between *Rhizobium* and *Azotobacter*, *Antonie van Leewenhoek, J Microbiol Serol*, **35** (1969) 533-40.
14. Sen S P, Energy recycling in biological nitrogen fixation: Problems and prospects, in *Frontiers of Research in Agriculture, Golden Jubilee Symposium*, Indian Statistical Institute, Calcutta, 1982.
15. Sadhukhan A & Sen S P, A molecular approach to plant taxonomy, in *Recent researches in plant sciences*, edited by S S Bir (Kalyani Publishers, New Delhi) 1979, 698-708; also, *Modern trends in plant taxonomy* (Vikas Publishing House, New Delhi) 1980.
16. Sen S P, Origin and evolution of life: Some unanswered questions, *Presidential Address, 72nd Indian Science Congress*, Lucknow, 1985.

## J J Shah

Our main interests are related to phloem, heartwood formation, plants with gums and gum-resins, rubber bearing guayule plant and the biology of Mahua (*Madhuca indica*).

### Phloem

Both pteridophytic and angiosperm phloem have been investigated. Phloem structure and development in 40 pteridophytes have been studied with emphasis on the cytological changes occurring during the development of the sieve cells, especially the process of nuclear degeneration. Presence of crystals in the degenerating nucleus of the developing sieve cells of *Blechnum orientale*, *Pityrogramma calomelanos*, *Pteridium aquilinum*; sieve plate in the rhizome sieve elements of *Cyathea gigantea*; proteinaceous mass formed by the dumping of spherules in *Araiostegia pulchra* and *Dennstaedtia appendiculata*; uncommon wall thickening in the sieve cells of *Pteris wallichiana*; branched sieve elements in some ferns and rod-like proteinaceous bodies in the sieve cells of *Lycopodium phlegmaria* have been reported. The morphology of the sieve area in 40 species of pteridophytes has been explored.

A detailed study on the structure, distribution, dimensional interrelationship

and evolutionary trends of secondary phloem components in the stem of 225 woody and herbaceous tropical dicotyledonous taxa of Polypetalae and Gamopetalae has been made. The study concerned three major aspects: (1) Anatomical information, (2) Statistical confirmation involving normal distribution analysis and trend analysis, and (3) Mathematical model assessing the relative advancement levels of the secondary phloem.

The mathematical model prepared indicates that herbaceous taxa in general have more specialized secondary phloem than woody members and that specialization of various components of secondary phloem has not progressed simultaneously.

### Heartwood and Sapwood

In more than 20 Indian timber trees, histochemical and histoenzymological changes that occur with the passing of time, in the axial and ray parenchyma cells of the sapwood resulting in biosynthesis of the polyphenols and the necrobiosis of these cells, have been characterized.

The boundary between sapwood and heartwood is characterized as the point at which parenchyma cells die and this boundary moves centrifugally with

---

INSA Senior Scientist, Biotechnology Centre, Department of Microbiology, M.S. University of Baroda, Baroda-390002, Gujarat; Residence : 35B, Jayanti Housing Society, Behind Jain Temple, Vallabh Vidyanagar, Baroda.



continued growth of the stem and the formation of new sapwood from the cambium.

The major changes occurring during heartwood formation are necrobiosis of the living axial and ray paranchyma cells of the sapwood and progressive accumulation of extractives in them.

The role of ethylene in heartwood formation was confirmed by using ethephon, an ethylene releasing chemical, and by the application of AVG (aminoethoxyvinyl glycine), an inhibitor of *in vitro* ethylene biosynthesis.

Also, the vitality of the wood parenchyma was ascertained employing neutral red, Evan's blue and Janus green B. It suggests that the terminal oxidase system of mitochondria appears to be functional in all the sapwood cells and some cells of sapwood-heartwood boundary, but it is not functional in the cells containing phenolics.

### Plants with Gums and Gum-Resins

The important Indian forest trees yielding gum/gum-resin of commercial promise have been investigated using light and electron microscopes to study the mode of development of gum/gum-resin producing cell system and to understand as to how and where the synthesis of secreted material takes place and how the secreted material is eliminated from the protoplast.

Meagre production yield is the major factor in limiting rational exploitation of some important gum/gum-resin bearing forest trees and in this regard our successful results with *Sterculia urens*, *Anogeissus latifolia*, *Bombax ceiba*, *Mangifera indica*, *Soymida fabrifuga*,

*Acacia senegal*, *Terminalia crenulata* and many other tropical trees are indicative of the fact that these taxa may be tapped for increased exudation by the judicious use of optimum concentration of ethephon.

We have worked out the structural and developmental aspects of economic plants like bajra, jowar, ginger, turmeric, mango-ginger and garlic. Besides undertaking the project on general vegetative and reproductive anatomy, the mechanisms of pollen pistil interaction in *Madhuca indica* and *in situ* localization of rubber in the parenchyma cells of *Parthenium argentatum* (guayule) with new histochemical techniques have been established by the use of fluorescence microscopy.

The future directions of our research efforts will be towards studying heartwood formation in gymnosperm wood, vitality of sapwood in some timber trees, longevity of phloem in dicotyledons, development of tapping techniques and induction of gum/gum-resin exudation in some important trees.

### Selected Publications

1. Shah J J & Kothari I L, Histogenesis of garlic clove, *Phytomorphology*, **23** (1973) 162-70.
2. Shah J J & Fotedar R L, Structure and development of phloem in the rachis of *Pteris longifolia* L, *Phytomorphology*; **24** (1974) 107-13.
3. Shah J J & Fotedar R L, Sieve-tube members in the stem of *Cyathea gigantea*, *Am Fern J*, **64** (1974) 27-28.
4. Kothari I L & Shah J J, Structure and organization of shoot apex of *Allium sativum* L. *Israel J Bot*, **23** (1974) 216-22.
5. Fotedar R L & Shah J J, Phloem structure and development in *Blechnum orientale* L, *Am Fern J*, **65** (1975) 52-60.
6. Setia R C, Parthasarathy M V & Shah J J, Structure of gum producing tissues of *Commiphora mukul*, *Ann Bot*, **41** (1977) 999-1004.

7. Shah J J & Nair M N B, Nuclear autolysis in the young sieve cells of some ferns, *Nucleus*, **21** (1978) 161-68.
8. Shah J J, Baqui S & Pandalai R C, Histochemical changes during transition from sapwood to heartwood in *Acacia nilotica*, *IAWA Bull*, **2**(1) (1981) 31-36.
9. Shah J J, Nair G M & Kothari I L, Ultrastructural changes in gum-resin ducts of the bark of *Commiphora wightii* (Arnott) Bhandari induced by mechanical injury, *IAWA Bull*, **3** (3-4) (1982) 185-92.
10. Shah J J, Nair G M, Thanki Y J & Kothari I L, Isolated sieve tubes in the fruit of *Coccinia grandis* (L) Voight (Cucurbitaceae), *Ann Bot*, **51** (2) (1983) 251-53.
11. Nair G M, Venkaiah K & Shah J J, Ultrastructure of gum-resin ducts in cashew (*Anacardium occidentale*), *Ann Bot*, **51** (1983) 297.
12. Chavan R R & Shah J J, Statistical approach for the understanding of phloem in 125 tropical dicotyledons, *Proc Indian natn Sci Acad*, **49B** (1983) 28-36.
13. Shah J J, Patel K R & Chavan R R, Isolated sieve tube(s)/elements in the barks of some Angiosperms, *IAWA Bull NS*, **14**(4) (1983) 225-63.
14. Shah J J & Pandya Soham, Vitality of wood parenchyma, *Curr Sci*, **53** (15) (1984) 782-85.
15. Venkaiah K & Shah J J, Distribution, development and structure of gum ducts in *Lannea coromandelica* (Houtt), Merril, *Ann Bot*, **54** (1984) 175-86.
16. Salma A Baqui & Shah J J, Histoenzymatic studies in wood of *Acacia auriculiformis* Cann during heartwood formation, *Holzforschung*, **39** (1985) 311-320.



## A K Sharma

The principal contributions of Sharma and his group during the last 37 years cover different facets of chromosome research. The researches have been oriented towards (a) innovation of methods for the study of physical and chemical nature of chromosomes and identification of chromosome segments, (b) chromosome study in relation to phylogeny and plasticity in their behaviour, (c) chemical basis of chromosomal control of differentiation, (d) analysis of principles underlying the effect of physical and chemical agents on chromosomes with their utilization both *in vivo* and *in vitro*, and (e) additional DNA sequences and their significance.

### New Techniques and Effects of Chemicals

Several new schedules have been worked out enabling analysis of chromosomes from any organ and identification of finer details. Of the innumerable techniques devised, mention may be made of the methods using esculine, isopsoralene and umbelliferone as well as orcein banding techniques for repeated DNA sequences.

One of the important findings is the demonstration of water alone as the pretreatment and mutagenic agent under certain specific conditions. Plant pigments too have been shown to induce

chromosome breakage and mutation. Chemical and physical principles underlying chromosome clarification and chromosome breakage have been resolved.

### Concept of Speciation

These methods have also enabled an analysis of the chromosomes of different groups of plants and their role in evolution. The systematic status of different genera of both monocotyledonous and dicotyledonous species have been assessed and their evolutionary pathways clearly delineated.

With the application of these techniques, later extensive studies on vegetatively reproducing species have led to the establishment of a new concept of speciation. The concept involves that in asexually reproducing species, the somatic tissue represents a chromosome mosaic in which the normal complement occurs in highest frequency. The altered complements enter into the participation of daughter shoots and new genotypes originate without involving the complicated act of fertilization.

### Chromosome Chemistry

Concomitant with these advances, methods have been devised for the analysis of the chemical nature of plant chromosomes *in situ*, including

---

Professor & Programme Coordinator, Centre for Advanced Study (Cell & Chromosome Research), Department of Botany, University of Calcutta, Calcutta-700019; Residence : Flat No. 2F(2), 18/3, Gariahat Road, Calcutta-700019.

demonstration of alkaline phosphatase activity in plant chromosomes *in situ*, the analysis of chemically differentiated nature with the aid of enzyme digestion and extraction techniques, the role of chromosomal proteins in nucleoplasmic transfer and the presence of RNA in plant chromosomes in certain phases of metabolism.

### **Induction of Cell Rejuvenescence and Control of Differentiation**

Methods have been devised for the analysis of the chromosomes in the differentiated or adult nuclei. The induction of cell rejuvenescence has been achieved utilizing different growth hormones as well as 2,4-dichlorophenoxy-acetic acid.

The polytenic constitution in the differentiated nuclei has been resolved and the deficiency of sugar moiety of nucleic acid has been proved to be responsible for its polytenic state. Polyteny has been shown to be a method adopted by chromosomes for the supply of fresh strand of DNA for uninterrupted transcription without involving cell division. According to this theory, in the mature organ the differentiation as such remains a continuous process despite the limited transcribing capacity of DNA molecule.

### **Concept of Chromosome Dynamism**

An analysis of the chemical nature of chromosomes in differentiated organs of plants through *in situ* estimation of DNA and protein as well as extraction and analysis has revealed that the chemical nature of chromosomes maintaining the basic DNA content varies from organ to organ, involving specially amplification of

DNA and difference in quantities of basic and non-basic proteins.

The above studies on chromosome structure and behaviour have revealed that increase in complexity in chromosome structure has been associated with the evolution of genetically controlled flexibility of their behaviour. The theory of chromosome dynamism thus explains the mechanism through which chromosomes exert control over phasic differentiation as well as reproduction and evolution.

### **Repeated DNA Sequences and Dynamic DNA**

Advances in techniques have also enabled analysis of the repetitive DNA sequence in plant chromosomes and their function. On that basis, despite the different epithets so far applied on these additional DNA sequences, the term dynamic DNA has been proposed based on their demonstration of the properties of amplification, dispersion and mobility. In addition to their non-specific functions, their controlling role in different gene controlled reactions has been elucidated.

In general, contributions of this group have led to new methods for chromosome analysis, establishment of a new theory of speciation, analysis of the chemical nature of plant chromosomes, suggestion of reorientation of taxonomy of angiosperms on genetic grounds, induction of cell rejuvenescence, demonstration of the chemical basis of chromosomal control of differentiation in plants, and establishment of the theory of chromosome dynamism and theory of dynamic DNA.

The Centre of Advanced Study which Sharma and his wife, Prof. Archana Sharma, have established is known as one



of the largest centres of chromosome research. The international journal "Nucleus" is being published from here for the last 27 years. Sharma has published almost 400 original articles.

Sharma has also worked on problems related to environment, originally emanating from his studies on genetic effects of physical and chemical agents. His Presidential Address at the 68th Session of the Indian Science Congress on "The Impact of the Development of Science and Technology on Environment" has provided incentives for the introduction of a number of programmes for conservation of environment and abatement of pollution in India.

### Selected Publications

1. Sharma A K, Trichloroacetic acid and Feulgen staining, *Nature, Lond*, **167** (1951) 441-42.
2. Sharma A K & Bhattacharjee Dipti, Effect of trichloroacetic acid on nuclear proteins, *Nature, Lond*, **169** (1952) 417.
3. Sharma A K, Mookerjee Archana & Ghosh Chitra, Alkaline phosphatase technique in plant chromosomes, *Port Acta Biol*, **3** (1953) 341-54.
4. Sharma A K & Sen Subir, Study of the effect of water on nuclear constituents, *Genet Iber*, **6** (1954) 19-32.
5. Sharma A K & Das N K, Study of karyotypes and their alterations in Aroids, *Agric Lus*, **16** (1954) 23-48.
6. Sharma A K & Roy Mira, Orcein staining and the study of the effect of chemicals on chromosomes, *Chromosoma*, **7** (1955) 275-80.
7. Sharma A K, Fixation of plant chromosomes, its principles, limitations and recent developments, *Bot Rev*, **22** (1956) 665-95.
8. Sharma A K, A new concept of a means of speciation in plants, *Caryologia*, **9** (1956) 93-103.
9. Sharma A K & Sharma (nee Mookerjee) Archana, Fixity in chromosome number of plants, *Nature, Lond*, **177** (1956) 335-36.
10. Sharma A K & Roy Mira, Chemical constitution and enzyme activity of chromosomes and related structures, *La Cellule*, **58** (1956) 109-33.
11. Sharma A K & Sharma Archana, A theory regarding the stability of chromosome complement in a species, *Naturwissenschaften*, **44** (1957) 1-17.
12. Sharma A K & Sharma (nee Mookerjee) Archana, Investigations leading to a theory of differentiation in plant cells, *Genet Iber*, **9** (1957) 143-62.
13. Sharma A K & Sharma Archana, Recent advances in the study of chromosome structure, *Bot Rev*, **24** (1958) 511-49.
14. Sharma A K & Sharma (nee Mookerjee) Archana, Chromosomal alterations in relation to speciations, *Bot Rev*, **25** (1959) 514-44.
15. Sharma A K, & Gupta S, Chromosome breakage with plant pigments, *Nature, Lond*, **184** (1959) 1821.
16. Sharma A K & Sharma Archana, Spontaneous and chemically induced chromosome breaks, *Int Rev Cytol*, **10** (1960) 101-36.
17. Sharma A K, Chaudhuri M & Chakraborty D P, Isopsoralene and its use in karyotype analysis, *Stain Technol*, **37** (1962) 95-97.
18. Sharma A K & Chatterjee A K, Amino-acid constitution of chromosomes, *J Histochem Cytochem*, **12** (1964) 266-70.
19. Sharma A K, Evolution and taxonomy of monocotyledons, *Chromosomes Today*, II (Oliver and Boyd, London) 1967, 2.
20. Sharma A K, Polyploidy and chromosome size, *Chromosomes Today*, Vol 3 (Oliver and Boyd, London) 1972 248-52.
21. Sharma A K & Sharma Archana, Chromosome techniques, in *Encyclopedia of microscopy and microtechnique*, edited by P Gray (Van Nostrand, New York) 1973, 77.
22. Sharma A K, Plant cytogenetics, in *The Cell Nucleus*, Vol 2, edited by H Busch (Academic Press, New York) 1974, 264-92.
23. Sharma A K, Chromosome banding and repeated DNA, *J Indian bot Soc*, **54** (1975) 1-8.
24. Sharma A K, A new look at chromosome and its evolution, *Proc Indian natn Sci Acad*, **42** (1976) 12-24.
25. Sharma A K, Additional genetic elements in chromosome, *Nucleus*, **21** (1976) 113-16.
26. Sharma A K, Change in chromosome concept, *Proc Indian Acad Sci*, **87B** (1978) 161-90.
27. Sharma A K & Banerjee M, Variations in DNA content, *Experientia*, **35** (1979) 42-43.
28. Sharma A K & Lavania U C, Trypsin-orcein banding in plant chromosomes, *Stain Technol*, **54** (1979) 261-63.
29. Sharma A K, Chromosome and distribution of monocotyledons in the Eastern Himalayas, in

- Tropical Botany*, edited by Larzonk (Academic Press, New York) 1979, 327-38.
30. Sharma A K, Sen H & Chaudhuri R K, DNA, RNA and protein content of isolated nuclei from different plant organs, *Indian J exp Biol*, **18** (1980) 1519-23.
  31. Sharma A K & Lavania U C, An interphase model for mitotic chromosome organization in eukaryota, *Biosystems*, **14** (1981) 171-79.
  32. Sharma A K, Additional genetic materials in chromosomes, *Kew Chromosome Conference II*, edited by P E Brandham and MD Bennett (George Allen and Unwin, London) 1983, 35-42.
  33. Sharma A K & Lavania U C, Mitotic spatial model: Arrangement of homologous chromosomes, *Genetica*, **62** (1984) 203-8.
  34. Sharma A K & Lavania U C, On the interchromosomal connections in plants, *Experientia*, **40** (1984) 94-95.
  35. Sharma A K, Dynamic DNA and chromosome structure, *Genetica: New Frontiers I, Proc XV Int Congress Genet*, 1983, 205-11.
  36. Lavania U C & Sharma A K, Arrangement of the interphase chromosomes in the nucleus, *J Hered* (1985) 76.
  37. Sharma A K & Sharma Archana, *Chromosome techniques—Theory and practice* (1st, 2nd and 3rd editions) (Butterworths, London) 1967, 1972, 1980.
  38. Sharma A K & Sharma Archana, *Chromosomes in Evolution of Eukaryotic Groups*, Vols I, II (CRC Press Inc, USA) 1983, 1984.



## Archana Sharma

In the tremendous advancements in the study of chromosome structure and function during the past 30 years, a major role has been played by improvements in technology. The research contributions of Archana Sharma relate to different aspects of chromosomal changes with reference to evolution, diseases, differentiation and senescence and the induction of such changes through different agents, principally environmental ones. The organisms surveyed include higher plants and animals and the human system.

### (A) Plant Chromosomes

The contributions of the group led by Prof. A K Sharma and herself on this aspect were, during the period 1950-1970, the most extensive in the area of chromosome research technology in plant groups in the world. Their joint publication, *Chromosome techniques—Theory and practice* (Butterworths, London) gained worldwide recognition. Their research publications resulting from studies conducted using these technological innovations covered most of the families of the plant kingdom, particularly the monocotyledons and aided in the analysis of the interrelationships, taxonomy and evolution of the different taxa. These intensive investigations led to the formulation of a theory for speciation in vegetatively reproducing plants, earlier

regarded as blind lanes in speciation. Another major aspect was the induction of division in differentiated tissue using the precursors and components of nucleic acids. It resulted in the explanation of differentiation of plant organs through differential duplication of the chromosomal segments, thus indicating the vital role of polyteny or endoreduplication in differentiation.

### (B) Human Chromosomes

After 1970, Mrs Sharma extended her interest to the study of human chromosomes following the advent of banding pattern techniques for the more specific identification of chromosome segments and their correlation with specific clinical syndromes. The work was carried out principally in collaboration with Geeta Talukder. Later, genetic markers were also analysed, with a view to assessing genetic polymorphisms in human populations. The following are the principal contributions:

(i) A study was made of genetic markers of human blood in tribal and non-tribal populations in West Bengal and the frequency of occurrence of polymorphic variants Hp, Tf, Lp, etc. was established. A new report of transferrin DChi was made from Santals of Midnapore.

(ii) Analysis of congenital malformations in different socio-economic groups from

---

Professor of Genetics, Centre for Advanced Study in Botany, University of Calcutta, Calcutta-700019;  
Residence : Flat No. 2F(2), 18/3 Gariahat Road, Calcutta-700019.

hospital records indicated the same frequency of congenital anomalies, in particular of chromosome aberrations, as in other more developed countries. No relationship could be recorded with maternal age.

(iii) Hyperlipidaemias have shown familial inheritance related to a certain extent to oil diet.

(iv) The frequency of beta thalassaemia and haemoglobin E was higher in eastern India, indicating a wide distribution of the mongoloid trait.

Another aspect studied is the relationship of human chromosomes and DNA content with phenomena like sex chromosomal abnormalities and ageing.

The DNA content of human sex chromosomes has been shown to be directly related to the number of "active" chromosomes, as suggested by the phenomenon of dosage compensation. DNA content per nucleus does not alter with age, but the number of chromosomes does, hypoploidy being observed in some cells of individuals above 60 years of age *in vivo*.

An association has been observed with DNA content and the level of differentiation of foetal human organs. DNA contents show ranges intermediate between 2C and 4C in different organs following tissue culture. These ranges differ for different organs. A theory of genetic programming, involving polyteny and fixed cell doubling, has been proposed to account for such variability in DNA content.

### (C) Environmental Mutagenesis

While surveying human populations for chromosomal anomalies, environmental

factors acquire considerable significance. A considerable part of the published work of Sharma and her later colleagues deals with the effects of environmental agents on laboratory test systems as well as on the general populations. The effect of different chemicals, including pesticides, insecticides and herbicides, on plant and animal systems *in vivo* and particularly on mammalian and human cells *in vitro*, has been examined. A thorough assessment has been made of the effects of almost all metals of the periodic table, single and in different combinations, on chromosome structure and cell division. In general, the clastogenic effects of metals have been found to be related directly to the period of exposure and the concentration used. Within each vertical group of the periodic table, cytotoxicity is directly proportional to increase in atomic weight, electropositivity and solubility of the cations in water and lipids. Within the horizontal group, cytotoxicity increases with successive periods. The interactions between metals, as well as the diet, modify the effects considerably. This work has special relevance in view of the increase in metals as components of industrial pollution—in screening for their threshold values and containment of harmful effects.

An allied aspect is the action of addictive substances like tobacco and betel quid in inducing cytotoxicity and their neutralization. Lime and betel leaf both tend to neutralize this action, as shown by changes in chromosomes and DNA content.

A survey of genetic markers and hospital records of congenital malformations of selected populations from West Bengal indicates a statistically higher level of genotoxicity in populations



from highly industrialized areas, as compared to those from rural and highly urbanized areas.

### Selected Publications

1. Hazra R R & Sharma A, Chromosomes of Indian Vitaceae, *Folia Biologica*, **18** (1970) 123-33.
2. Sharma A & Hazra R R, Chromosome studies on different species and varieties of *Sida* with special reference to accessory chromosomes, *Cytologia, Japan*, **36** (1971) 285-97.
3. Sharma A & Sanyal B N, Cytological studies on Indian Cyperaceae. I. Scirpeae, *Cytologia, Japan*, **37** (1972) 13-32.
4. Sharma A & Jash M, Cytology of some Indian members of Asclepiadaceae, in *Chromosome Today*, III (Oliver and Boyd, London) 1972, 248-52.
5. Sharma A & Ghorai A, Bambusae—A review, *Feddes Repertorium, Berl*, **81** (1980) 281-89.
6. Sinha S & Sharma A, Biology of *Lantana camara* L, *Feddes Repertorium*, **95** (1984) 621-33.
7. Roychowdhury S, Sharma A, Talukder G & Bhattacharyya D K, Acrylamide gel electrophoresis patterns of human alkaline phosphatase activity, a population study in Eastern India, *Gangenhaur's morph Jahrb, Leipzig*, **122** (1976) 603-7.
8. Ajmani M, Sharma A, Talukder G & Bhattacharyya D K, Genetic interaction of  $\beta$ -thalassaemia genes in population of Eastern India, *Indian J exp Biol*, **15** (1977) 455-57.
9. Thomas R, Sharma A & Talukder G, Transferrin patterns in Eastern India in normal and malignant cases, *Gann (Jap J Cancer)*, **68** (1977) 745-49.
10. Ajmani M, Sharma A & Talukder G, Haemoglobin variants in India, *Acta Geneticae Medicae Gemellologiae*, **27** (1978) 11-31.
11. Roychowdhury A, Talukder G & Sharma A, Total protein patterns in human amniotic fluid, *Aust J exp Biol med Sci*, **56** (6) (1978) 737.
12. Roychowdhury A, Talukder G & Sharma A, Genetic variations in proteins of the amniotic fluid, *Obstet Gynecol Surv, Baltimore*, **33** (1978) 627-40.
13. Mulherkar R, Sharma A & Talukder G, Chromosomal and cytochemical changes in human fibroblast culture—A review, *J scient ind Res*, **38** (1979) 322-27.
14. Bhattacharyya B, Talukder G & Sharma A, Study of genetic variants of serum hyperlipidaemias in Calcutta, *Indian J exp Biol*, **17** (1979) 656-58.
15. Talukder G & Sharma A, Genetic diseases in India—Present status, *Proc Indian natn Sci Acad*, **45** (1979) 273-306.
16. Sharma A, Talukder G & Singhania L, Prenatal diagnosis of genetic defects, *Curr Sci*, **52** (1983) 704-10.
17. Sharma A, Haemoglobinopathies in India, in *Peoples of India, Sp Publ XV International Congress of Genetics*, New Delhi, India, 12-21 December 1983, 31-49.
18. Sharma A & Talukder G, Genetical models of senescence in man, *Nucleus*, **22** (1979) 128-41.
19. Swain B K, Sharma A & Talukder G, Genetics of human IDH—A review, *Golden Jubilee Volume, Natn Acad Sci, India* (1980) 63-96.
20. Nigli M, Talukder G & Sharma A, Sex chromosomal abnormalities in India—A review, *Trop Geograph Med (Leiden)*, **32** (1980) 206-15.
21. Sen S, Sharma A & Talukder G, Relative DNA content in lymphocytes and buccal mucosa of patients with sex chromosomal anomalies, *Cytobios (Camb.)*, **42** (1985) 87-92.
22. Giri A K, Datta S R, Banerjee A, Sharma A & Talukder G, Transferrin variant DChi in tribals of Eastern India, *Human Heredity*, **35** (1) (1985) 56-65.
23. Sharma A, Tumour production in plants by plant extracts, *Nature, Lond*, **184** (1959) 1083-84.
24. Sahu R K, Basu R & Sharma A, Genetic toxicology testing of some plant flavonoids by the micronucleus test, *Mutat Res*, **89** (1981) 69-74.
25. Talukder G & Sharma A, Genetic disorders and environmental factors in the Indian background, *Proc III Int Conf Environmental Mutagens*, Tokyo, 1981, 33.
26. Chatterjee K K, Talukder G & Sharma A, Effect of synthetic pyrethroids on mammalian chromosomes, I. Sumicidin, *Mutat Res*, **105** (1982) 101-6.
27. Das A K, Sharma A & Talukder G, Effect of mercury-selenium antagonism on mammalian cell division, *Cytobios (Camb)*, **42** (1985) 271-73.
28. Giri A K, Talukder G & Sharma A, Mutachromosomal effects of tertbutylhydroquinone on bone marrow cells of mice, *Food Chem Toxicol*, **22** (6) (1984) 459-60.
29. Giri A K, Singh O P, Sharma A & Talukder G, Comparative effects of chronic treatment with

- certain metals on cell division, *Cytologia, Japan*, **49** (1984) 659-65.
30. Sharma A, Mukherjee A & Talukder G, Modification of cadmium toxicity in biological systems by other metals, *Curr Sci*, **54** (1985) 539-49.
  31. Sharma A, Higher plants as cytogenetic monitors for chemical agents, *J Indian bot Soc* (Birbal Sahni Medal Award Lecture), **64** (1985) 9-16.
  32. Sharma A, Metals as clastogens, *Presidential Address, Section of Biological Science, Nat Acad Sci, India*, (1985) 1-9.
  33. Sharma A K & Sharma A, *Chromosome techniques—Theory and practice* (Butterworths, London), First edition 1965, pp 474, reprinted 1967; Second edition 1972, pp 575; Third edition 1980, pp 711; Fourth edition, in press.
  34. Sharm A, *The chromosomes* (Oxford and IBH, New Delhi) 1976, pp 286, Second edition, in press.
  35. Sharma A, Talukder G & Mukherjee S K, *Methods in human genetics* (Kalyani Publishers, Ludhiana, National Book Trust) 1983, pp 350.
  36. Sharma A, Environmental chemical mutagenesis, *Perspective Rep Ser 6: Golden Jubilee Publications, Indian National Science Academy*, New Delhi, 1984, pp. 53.



## B N Singh\*

The discovery made about two decades back that small free-living aerobic soil amoebae belonging to the genera *Acanthamoeba* and *Naegleria* cause fatal human disease affecting the central nervous system, the former also causing human corneal ulcers leading to blindness, has revolutionized the very concept of parasitism by breaking down the barrier separating free-living organisms from parasites. The aetiology and epidemiology, as well as the control, of the diseases caused by soil amoebae are serious problems needing urgent investigation<sup>1-3</sup>

### Isolation and Culture of Small Free-living Amoebae

Extensive studies carried out by Singh on the selectivity of bacterial food by soil amoebae from 1941 onwards led to the use of non-nutrient agar and a suitable edible bacterium, such as *Klebsiella pneumoniae*, for the isolation and clonal cultivation of amoebae and amoeboid organisms from soil and other substrates, and for the count of their numbers in these substrates. These methods have been accepted universally as procedures of choice, and have been successfully employed by workers for the isolation and culture of environmental strains of pathogenic and non-pathogenic amoebae and also amoebae from human cerebrospinal fluid

and from human brain tissues postmortem from cases of primary amoebic meningo-encephalitis<sup>1,2,4-6</sup>.

### Classification of Amoebae

The recognition and classification of amoebae without test are problems which have hindered progress in gaining knowledge about organisms important on the one hand as components of soil and aquatic ecosystems and on the other as potential pathogens of humans and lower vertebrates.

Before 1976, two principal systems of classification of amoebae were recognized. The one developed by Singh<sup>7</sup> and Singh and Das<sup>8</sup> was based on mitotic patterns in amoebae. It throws light on the probable evolution of amoebae from flagellate ancestors. The other system propounded by Schaeffer and other taxonomists of pseudopodial school was based on locomotive form and pseudopodial characters in amoebae. Although these characters are important for generic differentiation, they are of no value in the creation of higher taxa based on probable evolution of amoebae<sup>9,10</sup>. Singh and Hanumaiah<sup>9</sup> and Singh<sup>10</sup> have combined both the systems into a single scheme. They have included the genera based on locomotive form and behaviour of amoebae in one of the families created by

---

Emeritus Scientist (CSIR), Central Drug Research Institute, Lucknow-226001; Residence : Room No. 205, Carlton Hotel, Lucknow.

\*Since deceased.

Singh<sup>7</sup> and Singh and Das<sup>8</sup> based on mitotic patterns. This scheme should enable workers to identify known and suspected pathogens, and to differentiate them from other species of amoebae.

### **Epidemiology of Human Meningo-encephalitis Caused by Naegleria**

The first isolates of environmental strains of *Naegleria aerobia* Singh and Das (*N. fowleri* Carter), causing fatal human meningo-encephalitis in different parts of the world, were reported by Singh and Das from five sewage sludge samples of Lucknow. Singh and Das showed that the temporary flagellate stage of two strains of *N. aerobia* (one from a fatal human case in USA and the other from a Lucknow sewage sludge) caused meningo-encephalitis and death of mice when given intranasally. Thus, the flagellate stage of *N. aerobia* seems to be the infective stage in the spread of human disease. The flagellates can get transformed into amebae on entering the human nose, the latter causing meningo-encephalitis. Amoebae cannot remain suspended in water for a long period in order to enter human nose<sup>2,3</sup>.

### **Primary Amoebic Meningo-encephalitis Caused by Acanthamoeba**

Gogate, Singh, Deodhar and Jhala have reported two acute fatal human cases of meningo-encephalitis caused by *Acanthamoeba* from Bombay. From one case, *A. rhysodes*<sup>7</sup> and from the other *A. culbertsoni*<sup>8</sup> were isolated from cerebrospinal fluid on non-nutrient agar seeded with *Escherichia coli*. Large number of amoebae were seen in the brain sections of both the patients postmortem. Both the species of amoebae were found to be pathogenic to mice when given

intranasally. Fatal cases of human meningo-encephalitis caused by *Acanthamoeba* had been reported earlier only from immunologic studies of sera or brain sections after tissue fixation and also on the basis of the presence of cysts<sup>3</sup>. So far, there is no record of *A. rhysodes* causing fatal human disease.

### **Excystation in Small Free-living Amoebae**

The biochemical basis of excystation in protozoa is not fully understood. An understanding of this process of differentiation is not only of academic interest, but has application in preventing human-beings from passing out cysts of *Entamoeba histolytica*, which are a source of infection in the spread of amoebiasis<sup>11</sup>.

Singh, Singh, Mathew and Sreenivasaya and others have shown that bacterial environment is necessary for the excystment of the cysts of ciliates and amoebae. Singh, Mathew and Anand<sup>12</sup> discovered that aqueous extract of bacteria caused nearly cent per cent excystment of cysts of a soil amoeba. A part of the excystment-inducing activity was due to the presence of amino acids, some of which have been identified. Certain chemically pure amino acids, at a suitable pH, also caused excystment. Singh, Datta and Dutta showed that both D- and L-forms of amino acids caused excystment of cysts of six species of small free-living amoebae to varying degrees at a pH of about 6.5. When a mixture of amino acids was used, the percentage excystment was much higher than that obtained with individual amino acids. The role of amino acids in the excystment of cysts of amoebae has been confirmed by several workers<sup>1,2,11</sup>.



### Virulence of *Entamoeba histolytica*

The nature of changes which occur when *E. histolytica* ceases to be a harmless lumen dweller and invades the mucosa is not understood properly. Various theories have been put forward to explain the differences between invasive and non-invasive strains<sup>2,11</sup>.

The discovery made by Singh and his colleagues in 1959 that attenuated and avirulent strains of *E. histolytica* from symptomless human carrier cases can be made as virulent as the strains from acute cases of human dysentery by feeding amoebae growing with bacterial associate with fine particles of cholesterol or by feeding rats with cholesterol and then inoculating avirulent strains intracaecally clearly shows that *E. histolytica* can change from virulent to avirulent state and vice versa<sup>1,2,11,13</sup>. The findings of Singh and his colleagues have been confirmed in the case of non-invasive or avirulent strains of *E. histolytica* growing either with bacterial associate or axenically<sup>11</sup>.

### Fruiting Myxobacteria

Fruiting myxobacteria have been found to follow two general modes of existence. Some of them are cellulolytic and the others are capable of attacking living or dead cells of true bacteria. They have also been shown to produce antibiotics against a variety of microorganisms<sup>14</sup>.

Singh<sup>15</sup> was the first to use non-nutrient agar and a species of bacteria, *Aerobacter* sp., for the isolation and culture of eubacteriolytic myxobacteria from soils and other substrates. This method has been considered as the procedure of choice for the isolation of eubacteriolytic myxobacteria and has been termed as 'Singh plate method' or the 'aerobacter

circle plate method'. A great variety of eubacteriolytic myxobacteria from Indian soils, bark of trees and dung of herbivorous animals have been isolated by Singh and Singh<sup>14</sup> using the 'aerobacter circle plate'.

### Selected Publications

1. Singh B N, Current status of the problem of exogenous and endogenous amoebiasis, *J scient ind Res*, **32** (1973) 399-434.
2. Singh B N, *Pathogenic and non-pathogenic amoebae* (Macmillan Press Ltd, London and Basingstoke), 1975.
3. Singh B N & Dutta G D P, Small free-living aerobic amoebae: Soil as a suitable habitat, isolation, culture, classification, pathogenicity, epidemiology and chemotherapy, *Indian J Parasitol*, **8** (1984) 1-23.
4. Singh B N, Culturing soil protozoa and estimating their numbers in soil, *Soil zoology* (Butterworths Scientific Publications, London) 1955, 403-11.
5. Singh B N, Interrelationship between micro-predators and bacteria in soil, *Proc 47th Indian Science Congress Ass, Part II*, 1960, 145-58.
6. Singh B N, Recent advances in soil protozoology, in *Proc 1st Summer School Zoology* (Manager of Publications, Government of India, Delhi) 1963, 79-103.
7. Singh B N, Nuclear division in nine species of small free-living amoebae and its bearing on the classification of the order Amoebida, *Phil Trans R Soc*, **B236** (1952) 405-62.
8. Singh B N & Das S R, Studies on pathogenic and nonpathogenic small free-living amoebae and the bearing of nuclear division on the classification of the order Amoebida, *Phil Trans R Soc*, **B252** (1970) 435-76.
9. Singh B N & Hanumaiah V, Studies on pathogenic and non-pathogenic amoebae and the bearing of nuclear division and locomotive form and behaviour on the classification of the order Amoebida (Monograph No 1, Association of Microbiologists of India), *Indian J Microbio* (1979) 1-80.
10. Singh B N, Nuclear division as the basis for the possible phylogenetic classification of the order Amoebida Kent 1880, *Indian J Parasitol*, **5** (1981) 133-53.

11. Singh B N, Present state of our ignorance concerning amoebiasis caused by *Entamoeba histolytica*, *Indian J Parasitol*, **6** (1982) 1-11.
12. Singh B N, Mathew S & Anand B, The role of *Aerobacter* sp, *Escherichia coli* and certain amino acids in the excystment of *Schizopyrenus russelli*, *J gen Microbiol*, **19** (1958) 104-11.
13. Singh B N, Effect of cholesterol on the virulence of *Entamoeba histolytica* in rats, *J scient ind Res*, **18C** (1959) 166-69.
14. Singh B N & Singh N B, Distribution of fruiting myxobacteria in Indian soils, bark of trees and dung of herbivorous animals, *Indian J Microbiol*, **11** (1971) 47-92.
15. Singh B N, Myxobacteria in soil and composts: Their distribution, number and lytic action on bacteria, *J gen Microbiol*, **1** (1947) 1-10.



## J S Singh

The work of Singh has been mostly on grasslands and forests.

### Grasslands

Analysis of canopy architecture revealed that depending on the growth form, the pattern of vertical distribution of biomass among species is variable, as is their temporal growth pattern. Additionally, different layers of vegetation are dominated in different months by different species according to their light- and water-adaptation characteristics. Integration of such varied species, often belonging to different "guilds", into a community, results in a multilayered, dynamic canopy in which each quantum of incident light has greater probability of being intercepted and used than in a single-layered canopy.

Analysis of vegetation across different bioclimates indicated evolutionary-adaptational response of root-shoot ratio to water availability. In low rainfall areas, grassland vegetation tends to increase its photosynthetic canopy as and when enough water is available. The root system is shallow and acts mostly as surface feeder, absorbing the water quickly before it is evaporated following a rainfall event. In medium rainfall areas, competition for soil water is intense because of a general stimulation of growth; therefore, some increase in rainfall would result in increase in the percentage of root material. In high

rainfall areas, a balance is attained between competition for soil water and light, resulting in an asymptote for the percentage of root material. The relationship between below-ground plant biomass and live shoot biomass in grasslands was found to be sigmoid. The grasslands in the vicinity of the plateau of the curve would be more exploitable in terms of herbage, while the others would be more sensitive to grazing pressure. The grasslands of dry region need more energy to pump similar amounts of nitrogen in the biological system as compared to those of the humid regions. In contrast, the humid grasslands cycle lower quantities of phosphorus in effecting fixation of comparable amounts of energy.

Species composition was demonstrated to be a significant factor influencing the primary productivity of grasslands located within the same climatic regime. Concurrent studies on primary productivity, decomposition and soil respiration indicated that up to about 35% of the decomposed plant debris in successional grasslands is conserved as new soil organic matter; and accumulation of this energy may provide a motive for succession towards a woodland under protection from grazing. Simulation-optimization modelling showed that in the long term, moderate grazing punctuated by a light-grazing year every 4-5 years for

the legumes to recoup, is the best management strategy for the sub-humid Indian grasslands.

Methods for the measurement of primary productivity and functional root biomass were standardized. Simulation of root biomass and sampling regimes indicated that the random errors associated with sampling of roots in commonly used methods may invalidate the production estimates. Properties underlying the stability and resilience, and limits of tolerance of grasslands to various stresses need to be categorized and quantified.

## Forests

Analysis of the vegetation of the rain-forest eco-systems of the Silent Valley revealed the preponderance of woody vegetation and species with leaves in mesophyll size class, pronounced diversity and multilayering with abundance of epiphytes and stranglers, and a strong tendency to change in species composition in time and space. In contrast, the dominance-diversity curves for the Himalayan forests followed a geometric series conforming to the niche pre-emption situation in communities of low diversity.

Analysis of satellite images for the central Himalayan region revealed that a total of 27.8% of land area is covered with forest, and only 4.4% with a forest of > 60% crown density. A method was developed to quantify the forest biomass through aerial photographs and it was estimated that the total carbon stored in the central Himalayan forests and the annual net uptake of carbon are  $241.8 \times 10^{12}\text{g}$  and  $11.8 \times 10^{12}\text{g}$ , respectively. The net primary production in these forests ranges from 12.6 to 27.6 t ha<sup>-1</sup> and the

litter fall from 4.1 to 7.6 t ha<sup>-1</sup>. Because of the deforestation the Himalayan forests have become a net source of carbon for the atmosphere.

The catchments in the middle mountains were demonstrated to be subsurface-flow systems, with the major pathway of soil loss from these systems being landsliding. The pattern of succession after landslide emphasizes the importance of the initial floristic composition and the local propagule reservoir, and can be explained fully only when the holistic and reductionist approaches are considered together.

Analysis of the population structure of the Himalayan forests indicated that the potential natural vegetation of oaks is being replaced by early successional, light-demanding species, such as pine. Lopping, seed predation, grazing and change in the rainfall regime have also been implicated in the failure of regeneration of oak species. Studies on the nutrient cycling revealed that the high N efficiency of litter production and the high C-N ratio of litter in pine lead to immobilization of available soil N, making the habitat inimical to species (e.g., oaks) demanding higher amounts of N. Temperature and moisture, among abiotic variables, and lignin and N among substrate quality parameters, govern the rate of nutrient release in these forests, and precipitation components add significantly to the nutrient capital. Phenological activities, e.g., leaf drop, leafing, flowering and fruiting of a number of central Himalayan forests were quantified. These activities reflect an adaptation such that the advantages of the monsoon pattern of rainfall are fully utilized, and the possible nutrient leakages are minimized. In some forests, the



epiphytic vegetation contributes to total community chlorophyll as much as or greater than the combined contribution of herb and shrub layers.

Analysis of energy flow relationships between agro-ecosystems and forest ecosystems indicated that for each unit of energy harvested in the agronomic production in the Himalaya, 7 units of energy are expended from the forest ecosystem in terms of fuel, fodder and leaf manure; consequently, the carrying capacity of the forests has been far exceeded.

The impact of deforestation and conversions on the nutrient cycling characteristics of the ecosystems, the interactions between C, N, P and S cycles and the reproductive biology of important tree species need to be investigated.

### Selected Publications

1. Mehra M S, Pathak P C & Singh J S, Nutrient movement in litter fall and precipitation components for Central Himalayan forests, *Ann Bot*, **55** (1985) 153-70.
2. Negi K S, Rawat Y S & Singh J S, Estimation of biomass and nutrient storage in a Himalayan moist temperate forest, *Can J For Res*, **13**(6) (1983) 1185-96.
3. Pandey A N & Singh J S, Mechanism of ecosystem recovery: A case study from Kumaun Himalaya, *Reclam Reveget Res*, **3**(4) (1985) 271-92.
4. Pandey U & Singh J S, Nutrient changes and release during decomposition of leaf-litter in an Himalayan Oak-conifer forest, *Can J Bot*, **62**(9) (1984) 1824-31.
5. Pathak P C, Pandey A N & Singh J S, Apportionment of rainfall in Central Himalayan forests (India), *J Hydrol*, **76** (1983) 319-32.
6. Singh J S & Coleman D C, Distribution of photo-assimilated carbon-14 in the root system of a shortgrass prairie, *J Ecol*, **62** (1974) 389-95.
7. Singh J S & Joshi M C, Ecology of semiarid region of India with emphasis on land use, in *Management of semiarid ecosystems*, edited by B H Walker (Elsevier Scientific Publishers, Amsterdam) 1979, 243-75.
8. Singh J S, Trilica M J, Risser P G, Redmann R E & Marshall J K, Autotrophic subsystem, in *Grasslands, systems analysis and man*, edited by A Breymer and G M van Dyne (Cambridge University Press) 1980, 59-200.
9. Singh J S & Yadava P S, Seasonal variation in composition, plant biomass, and net primary productivity of a tropical grassland at Kurukshetra, India, *Ecol Monogr*, **44** (1974) 351-76.
10. Singh J S & Misra R, Diversity, dominance, stability and net production in the grasslands at Varanasi, *Can J Bot*, **47** (1969) 425-27.
11. Singh J S & Singh S P, Forest vegetation of the Himalaya, *Bot Rev*, **52**(3) (1986), in press.
12. Singh J S, Singh S P, Saxena A K & Rawat Y S, India's Silent Valley and its threatened rain forest ecosystems, *Environ Conserv*, **11** (1984) 223-33.
13. Singh J S & Gupta S R, Plant decomposition and soil respiration in terrestrial ecosystems, *Bot Rev*, **43** (1977) 449-528.
14. Singh J S, Pandey Uma & Tiwari A K, Man and forests: A Central Himalayan case study, *Ambio*, **13**(2) (1984) 80-7.
15. Singh J S, Lauenroth W K & Steinhorst R K, Review and assessment of various techniques for estimating net aerial primary production in grasslands from harvest data, *Bot Rev*, **41** (1975) 181-232.
16. Singh J S, Lauenroth W K, Heitschmidt R K & Dodd J L, Structural and functional attributes of the vegetation of northern mixed prairie of North America, *Bot Rev*, **49**(1) (1983) 117-49.
17. Singh J S, Lauenroth W K, Hunt H W & Swift D M, Bias and random errors in estimators of net root productions: A simulation approach, *Ecology*, **65**(6) (1984) 1760-64.
18. Singh J S, Rawat Y S & Chaturvedi O P, Replacement of oak forest with pine in the Himalaya affects the nitrogen cycle, *Nature, Lond*, **311** (1984) 54-6.
19. Singh J S, Tiwari A K & Saxena A K, Himalayan forests: A net source of carbon for the atmosphere, *Environ Conserv*, **12** (1985) 67-69.
20. Swartzman G L & Singh J S, A dynamic programming approach to optimal grazing strategies using a successional model for a tropical grassland, *J appl Ecol*, **11** (1974) 537-48.

## S K Sinha

After doing his M Sc in Botany from Agra College, Agra, Sinha joined as a lecturer in botany in DAV College, Kanpur. He specialized in cytogenetics and plant breeding and did work on the response of barley and linseed to the interaction of nitrogen and phosphorus in relation to pH at the Agricultural College, Kanpur. This work fetched him PhD degree of Agra University. In 1962, he left for Canada to join the University of Alberta, Edmonton, to work on the biosynthesis of amino acids in plants. He got his second PhD degree in plant biochemistry from the University of Alberta. He is currently Professor of Eminence (Plant Physiology) at the Water Technology Centre, Indian Agricultural Research Institute. His major contributions are discussed below in brief.

### (1) Plant Nutrition

Sinha's work on the interaction of nitrogen and phosphorus in relation to yield in barley and linseed included studies on seed quality, particularly the oil content and reproductive capacity of plants.

### (2) Amino Acids Biosynthesis

He has worked on the interconversion of glycine and serine, and the importance of C<sub>1</sub> compounds in the synthesis of serine and methionine. The splitting of glycine and its carbon-2 becoming carbon-3 of serine was first demonstrated by Sinha and

Cossins. This reaction subsequently became the basis of photorespiration. They also showed that exogenously supplied metabolites do not necessarily exhibit the same metabolism as those generated *in vivo*, due to compartmentation. In addition, the occurrence of transaminases with varying specificity to different amino acids was demonstrated.

### (3) Tuberization and Cyanogenic Glucosides in Cassava

It was demonstrated that the roots of cassava differentiate into tubers only after initiation of secondary growth. The development of tubers was strongly correlated with leaf area during the dry months at Trivandrum. Cyanogenic glucosides occur in all parts of the plant and the content in the edible portion is not related to any other tissue. Each tissue synthesizes its own glucosides. The bitter taste of the tuber is not indicative of the content of cyanoglucosides, because it could be influenced by the free sugar content.

### (4) Physiological and Biochemical Basis of Heterosis

Heterosis is generally considered a phenomenon through which better growth and yield are obtained, but no suitable physiological and biochemical basis is known. Sinha and colleagues proposed a



physiological complementary and multiplicative hypothesis to explain increased growth and yield in heterotic hybrids compared to their parents. For this, the hybrid need not have a higher activity of enzymes than its parents, but a combination of a series of enzymes would provide the advantage. The analysis, therefore, should be done by splitting a character into its components. Accordingly, the components of such a character as photosynthesis were identified for the genetical studies.

#### **(5) Physiological and Biochemical Analysis of Yield in Pulses**

It was demonstrated by Sinha and coworkers that most pulses produce a large number of flowers, but only a small percentage of them set fruits. When fruit development commences in most pulses, it is accompanied by either degradation or poor activity of nodules. There is a decrease in photosynthesis rate coupled with loss of RuBP carboxylase. Therefore, an intraplant competition hypothesis was proposed. More recently, however, it has been recognized that an appropriate balance in vegetative and reproductive growth can be achieved by adjusting the date of planting, population density and irrigation, which could help in yield improvement. Accordingly, it has been demonstrated that as against the prevailing concept of October planting, late November planting combined with irrigation leads to better productivity of gram (chickpea). However, irrigation can often lead to excessive vegetative growth in chickpea. Selection against this character is a desirable objective.

#### **(6) Photosynthesis Research**

Sinha demonstrated the value of reproductive organs as photosynthetic structures. Efforts were made to study the basis of more dry matter production in some chickpea mutants in comparison with their parents. These mutants did not differ in RuBP carboxylase/oxygenase activity,  $K_m$  or  $V_{max}$ , but the main difference was in leaf area development. Genetic studies on the components of photosynthesis have been made.

#### **(7) Physiology of Wheat**

Physiological analysis of tall, medium and dwarf wheats has been done. Since the first decade of this century, no improvement in dry matter has occurred, though yield has improved. High yield is not achieved because of the death of tillers. Differentiation of different genotypes has been studied to explain the pattern of growth and yield. It has also been shown that water use efficiency of wheat is as high as, or even better than, that of  $C_4$  plants when it is grown in its environment of adaptability.

#### **(8) Drought Resistance**

Through long-term experiments, the stability of different genotypes has been evaluated. The stable varieties, such as C 306, are good in a poor environment (less than 2.0 tonnes  $ha^{-1}$ ), but do not respond if the environment is good. Other properties of these cultivars are being studied. A hypothesis has been proposed according to which it is not possible to combine high yield with high drought tolerance. The hypothesis also explains as to why the seedling resistance is not necessarily maintained at the time of grain development. It is now being extended to pulses by manipulating 'sink' capacity.

Sinha is a Fellow of the Indian National Science Academy, the Indian Academy of Sciences and the National Academy of Sciences. He has been a recipient of the FICCI Award (1983) and the VASVIK Award (1985) in Agricultural Sciences.

### Selected Publications

1. Sinha S K & Khanna R, Physiological, biochemical and genetic basis of heterosis, in *Advances in Agronomy* (Academic Press, New York), 1975, 122-74.
2. Sinha S K, *Food legumes : Distribution, adaptability and biology of yield* (FAO, Rome) 1977.
3. Sinha S K & Nicholas D J D, Nitrate reductase in relation to drought resistance, in *Physiology and biochemistry of drought resistance*, edited by L G Paleg and D Aspinall (Academic Press, Sydney) 1981, 145-69.
4. Sinha S K & Swaminathan M S, New parameters & selection criteria in plant breeding, in *Contemporary basis of crop breeding*, edited by P B Vose and S G Blixt (Pergamon Press, Oxford) 1983, 1-35.
5. Sinha S K, Aggarwal P K & Khanna-Chopra R, Physiological and phenological basis of irrigation in India, in *Advances in Irrigation*, Vol 3 (Academic Press, New York) 1985, 129-212.



## H S Sohi

During the course of the last three decades (1954-1984), I have been associated with various plant pathological research projects at the Department of Botany, Panjab University, Chandigarh (1979-1982); IARI, New Delhi (1954-1962); Department of Plant Pathology, H P Agricultural College, Solan (1962-1969); Indian Institute of Horticultural Research, Bangalore (1969-1979 and 1982-1984); IARI Regional Station, - Amar Tara, Shimla (1984); and National Centre for Mushroom Research and Training, Solan (HP) (July 1984 to date). While conducting taxonomic studies on the myxomycetes of Mussoorie Hills, more than 100 species belonging to 40 genera were described. Majority of them were new records for India. Three new species were reported in Mycologia. From subsequent studies on diseases of fruits, vegetables and ornamental plants at Solan, Bangalore and Chandigarh, more than 30 fungal diseases infecting various economic host plants were reported as new records from India. About a dozen new species of plant pathogenic fungi were described. A new blight disease of mango (*Macrophoma mangiferae*) was studied intensively at Delhi and its perfect stage was obtained in culture for the first time. I carried out detailed investigations on rust disease of sugarcane (*Puccinia erianthi*). *Erianthus munja* was found to act as a collateral host. Teleutospores produced only at low temperature showed

germination. The pathogen could perpetuate at mid-hill elevations. I introduced the artificial cultivation of European mushroom in India and various synthetic compost formulae were developed at Solan. Strain 11 was selected for general cultivation based on its superior yield performance. Training was imparted to scientists, growers and extension workers for popularizing mushroom culture. This led to the establishment of various research centres and mushroom units in the country, especially in Himachal Pradesh. Its cultivation was extended to South India during 1970 from IIHR, Bangalore. A large number of fleshy fungi were collected during the course of surveys in the hills and were described. About a dozen of these were found to be edible. Attempts were made to cultivate guchchi (*Morchella* spp.) without success. This work encouraged many scientists to initiate systematic studies on fleshy fungi in different regions of the country.

I worked on red rot and smut diseases of sugarcane. Comprehensive studies were conducted on buck eye rot (*Phytophthora nicotianae* var *parasitica*), *Septoria* leaf spot (*S. lycopersici*) and powdery mildew (*Oidium* sp.) of tomato, fruit rot of bell pepper and brinjal (*Phytophthora nicotianae* var *capsici*), angular leaf spot (*Phaeoisariopsis griseola*), rust (*Uromyces*

phaseoli) and anthracnose (*Colletotrichum lindemuthianum*) of french bean, *S. petoria* leaf spot (*S. vignicola*), Anthracnose (C.I), rust (*Uromyces vignae*), ashy stem blight (*Macrophomina phaseolina*) and powdery mildew (*Erysiphe polygoni*) of cowpea, leaf spot of sapota (*Phaeophleospora indica*); downy mildew of balsam and poppy (*Peronospora obdusca* and *P. papavericola*), leaf blotch (*Alternaria porri*) and basal rot (*Sclerotium rolfsii*) of onion, leaf spot of balsam and chillies (*Helminthosporium* spp.), damping off of vegetables and ornamental plants (*Rhizoctonia solani*, *Macrophomina phaseolina*, *Pythium* spp. and *Phytophthora parasitica*) and cercospora leaf spots of different crops. In all these cases, life histories, factors affecting disease initiation and development, losses caused, evaluation of germplasm and control through cultural and chemical means were worked out. Practical and economical chemical control measures were worked out against hill bunt of wheat; black scurf of potato; buck eye rot, septoria leaf spot, powdery mildew and fruit rot of tomato; fruit rot and anthracnose of bell pepper; cercospora leaf spot of brinjal and chillies, powdery mildew, leaf spots, blight and rust of cowpea; damping off of cauliflower and ornamental plants; leaf blight of maize; rhizome rot and leaf spot of ginger; powdery mildew and rust of peas; leaf spot of sapota; fruit rot of guava; downy mildew, powdery mildew and anthracnose of grapes; anthracnose and powdery mildew of mango and papaya; anthracnose and powdery mildew of cucurbits; downy mildew and white rust of crucifers and rhizoctonia root rot and wilt of beans. Most of the recommendations which emerged from this work are being followed throughout the country. The role of

various cultural practices, including time and method of sowing, mixed cropping, irrigation and mulching, in disease incidence in different crops was studied. Some of these provided excellent control, e.g. staking of plants and mulching of plots against buck eye rot in tomato; proper pruning against mildews of grapes; disposal of diseased leaves and fruits against leaf spot of sapota and guava fruit rot; and adjustment of time of sowing against powdery mildew disease of peas and cowpea.

Bacterial wilt of solanaceous crops (*Pseudomonas solanacearum*) was investigated thoroughly. This strain was different from potato strain. All the commercial varieties of tomato and brinjal, except Pusa purple cluster, were susceptible, as also most of the resistant varieties introduced from abroad. Dingras Multiple Purple, Dumaguete and Sinampiro of brinjal and CRA, 66A selection of tomato were found resistant and were used in breeding programmes. Twelve to fourteen times reduction in yield of brinjal was noticed. A number of weed hosts (*Lagascia mollis*, *Solanum khasianum*) were found to act as alternative hosts. Sterilization of seed beds with formaldehyde (2%) or DD was found to eradicate soil infection. Different crop rotations were tried to reduce the incidence of the disease. Sequences where maize or ragi crops were used are more effective in reducing disease incidence. In the studies on pre-emergence damping off of ornamental plants, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Phytophthora parasitica*, *Pythium aphanidermatum* and *P. butleri* were found mostly associated. *R. solani* was more prevalent during winter, whereas *M. phaseolina* was more destructive during summer at Chandigarh.



They were pathogenic singly on amaranthus, balsam, candituft, calendula, chrysanthemum, delphinium, phlox, pansy, sweet pea, gomphrena, coxcomb, stock, zinnia, lupinus and daisy. In combination, they were more virulent in lathyrus and iberis. *R. solani* alone was more pathogenic on impatiens, lupinus and coxcomb and the latter on viola, daisy and gomphrena. The relative efficacy of 12 different fungicides was tested as seed dressers on different hosts. Bavistin, Benlate, PCNB, Thiram, Blitox and Difolatan, in general, provided very effective control of damping off by these pathogens. Similar studies were conducted in different vegetables and proper seed treatments were recommended. Detailed studies on market diseases of fruits and vegetables were conducted at Bangalore and Chandigarh. Seventy fungal diseases affecting 18 different fruits and 71 diseases on 35 vegetables were reported from Chandigarh. Quite a number of these were serious and new records. Their incidence and losses caused were investigated along with control measures. In the studies on inheritance of disease resistance in rust of peas in crosses between Bonneville (susceptible) with PJ 222117, 207508 and E C 109188 (resistant) parents, resistance was controlled by a single dominant gene. Similar studies were conducted in cowpea and capsicum. Detailed studies were conducted on seed-borne diseases of cowpea, watermelon, okra, peas, onion, bottlegourd, beans and different ornamental plants. Location of pathogen on/in seed, loss of viability and germination and other adverse effects were studied along with control measures. Because of the dangers of fungicide pollution and residual toxicity as a result of repeated field spraying, the efficacy of

various systemics when applied as seed dressers or soil drench on disease appearance and intensity was studied in cowpea, beans, okra and chillies. Indication of delayed appearance of powdery mildew, anthracnose and septoria leaf spot diseases were obtained. More than a dozen varieties of cowpea resistant to septoria leaf spot and rust diseases were bred. One of these is being grown by farmers in Karnataka state. Losses caused by important diseases in fruits and vegetables were worked out by artificially creating different disease intensities. Germplasms of grapevine resistant against mildews, anthracnose and rust diseases; of guava against anthracnose and fruit rot; of tomato against buckeye rot, septoriosis and powdery mildew; of pea against powdery mildew and rust; of beans against rust; of cowpea against septoria leaf spot, powdery mildew, ashy stem blight, rust and anthracnose; of capsicum against powdery mildew, anthracnose, cercospora blight and fruit rot; of onion against basal rot; of french bean against angular leaf spot and floury mildew; of rose against powdery mildew and tar spot; and of balsam against downy mildew were screened. Resistant varieties in each case were reported for use by breeders. I provided necessary pathological support to the breeders in the projects for breeding disease resistant varieties.

I have published about 220 scientific papers, including invited articles, status papers and bulletins. I have been President of Indian Society of Mycology and Plant Pathology for 1983 and Vice-President of the Indian Mycological Society, Calcutta, for the last six years.

Since July 1984 I am working as Officer on Special Duty at the National Centre for

Mushroom Research and Training at Solan (HP). I am also working as Coordinator for the All India Coordinated Mushroom Project. The National Centre is mainly devoted to undertaking research on various aspects of mushroom culture in India.

## Selected Publications

1. Sohi H S, The myxomycetes of the Mussoorie Hills—IV, *Mycologia*, **49**(1) (1957) 128-33.
2. Sohi H S, Studies on storage rot of mango caused by *Colletotrichum gloeosporioides* Penz and its control, *Phytopath Medit*, **12** (1973) 114-16.
3. Sohi H S, The perithecia producing strain of *Macrophoma mangiferae*, *Indian Phytopath*, **14** (1961) 98-99.
4. Sohi H S, Studies on blight disease of mango caused by *Macrophoma mangiferae*, *Indian Phytopath*, **13**(2) (1960) 137-43.
5. Sohi H S, New records of fleshy fungi from Himachal Pradesh, *Indian J Bot*, **44** (1965) 69-74.
6. Sohi H S, Growing of the common European mushroom *Psalliota bispora* in Himachal Pradesh, *Indian J Hort*, **22** (1965) 365-69.
7. Sohi H S, Diseases of crops, vegetables and subtropical fruits, *Farmers Bull*, **21** (1965) 1-34.
8. Sohi H S, New records of fungi from Himachal Pradesh-IV, *Punjab Univ Res Bull*, **23**(1,2) (1972) 109-11.
9. Sohi H S, New disease problems of fruit and vegetable crops in South India, *Second International Symposium in Plant Pathology*, Delhi, 1971.
10. Sohi H S, Morphological, cultural and physiological studies on *Septoria lycopersici* Speg, *Indian Phytopath*, **26** (1973) 666-73.
11. Sohi H S, Studies on spawn production in *Psalliota bispora* (Lang) Moll, *Indian J Microbiol*, **13** (1973) 54-56.
12. Sohi H S, Anthracnose in tropical fruits, *Dr R N Tandon Birth Day Celebration Volume*, February 1975.
13. Rao M V B & Sohi H S, Additional hosts for *Pseudomonas solanacearum* Smith, *Curr Sci*, **45**(2) (1976) 75-76.
14. Satyanarayana A, Rajinderan R, Rawal R D & Sohi H S, Inheritance of rust resistance in cowpea caused by *Uromyces phaseoli* var *vignae*, *Proc Twentieth Int Hort Congress*, 15-23 August 1978, Sydney.
15. Sohi H S, *Phytophthora* diseases of citrus and their control, *Status paper*, *Symposium on Phytophthora diseases of tropical cultivated plants*, CPCRI, Kasargod, 19-23 September 1980.
16. Sohi H S, Studies on fungal diseases of Salanaceous vegetables and their control, *Paper presented in the symposium convened by the Mycological Society of India*, Calcutta, September 1979.
17. Sohi H S, Present status of paddy straw (*Volvariella* spp) mushroom cultivation in India & future prospects, *Invitational paper presented in the 11th annual convention of Indian Soc Mycology*, Trivandrum, 23-24 February 1984.
18. Sohi H S, Diseases of ornamental plants in India & their control, *Scient Bull*, ICAR, New Delhi, 1984, in press.
19. Sohi H S, Present status of our knowledge of important fungal diseases of important vegetables in India & future needs, *Presidential address*, *Indian Soc of Mycology & Pl Pathology*, 23 May 1984, Solan.



## K R Surange

After doing his MSc in botany in 1943, Surange took to research under Prof. Birbal Sahni, FRS and obtained PhD degree in 1947. In 1946, he joined Botany School, Cambridge, UK, and carried out research under Prof H H Thomas, FRS as 1851 Exhibition Scholar. He obtained his second PhD degree from the Cambridge University in 1949. On his return to India, Prof. Sahni appointed him Reader in the newly founded Institute of Palaeobotany at Lucknow. Tragic events followed and Prof. Sahni died within a week of the laying of the foundationstone of the Institute of his dream. Surange became Assistant Director of the Birbal Sahni Institute of Palaeobotany in 1952 and then Officer-in-Charge in 1953. He was appointed Director in 1959 and held this position till his retirement in 1980. He also held the post of Director, Maharashtra Association for the Cultivation of Science (MACS), Pune from December 1980 to February 1985.

### Living Plants

Surange started his research career with morphological and anatomical work on the small South American family, Cyclanthaceae, and its fossil counterpart present in the Deccan Intertrappean Series. He carried out detailed morphological and anatomical studies on the species of *Carludovica* and *Cyclanthus*; the results were published in

the *Transactions of the National Institute of Sciences, India*.

### Fossil Plants

#### (1) *Coenopteroid Ferns*

Surange has made significant contributions on the Lower Carboniferous Coenopteroid ferns *Botryopteris* and *Stauropteris* from England. His investigations demonstrated two types of stems in the primitive *Botryopteris*, the trailing underground stem which at intervals gave rise to aerial stems, almost identical anatomically, and which bore highly branched dorsiventral structure of a "frond". In the evolution of lamina by webbing of ultimate branches, such "fronds" as those of *Botryopteris* might have served as intermediary stages.

The heterospory in the Lower Carboniferous *Stauropteris burntislandica* was a major discovery made by him in the early land plants; the results were published in the *Transactions of Royal Society, London*. One big mature and three aborted megaspores were contained in a gland-like vesicle. This led to the hypothesis that seed habit in plants in the later geological periods might have developed through such a structure as displayed by megasporangium of early *Stauropteris*.

---

Formerly, Director, Birbal Sahni Institute of Palaeobotany, Lucknow; Residence : B-20, Sector A, Mahanagar, Lucknow-226006.

Surange has also to his credit a monograph on the Indian Fossil Pteridophytes published by CSIR.

## (2) *Glossopteris* Flora

Surange and his students carried out extensive studies on the fossil plants comprising the *Glossopteris* flora of India, which gave rise to our major coal reserves.

In addition to a number of other fossil plant species, Surange and his school made important contributions to the knowledge of the widely spread and dominant form genus, *Glossopteris*. Several new reproductive organs, both male and female, belonging to Glossopteridales, were discovered and described in detail; this work demonstrated convincingly that a new class of gymnosperms was flourishing in the southern hemisphere, which consisted of different orders, families and genera, occupying the huge supercontinent of Gondwanaland throughout the Permian period.

Maximum species of *Glossopteris* are widely distributed in the coal-bearing horizons in India and other Gondwana countries. They could be of great use for deriving stratigraphical correlations. Unfortunately, the speciation of *Glossopteris* was so confusing that their correct identification was not always possible. Surange and his colleagues have demonstrated that venation pattern of *Glossopteris* leaves could be used as "fingerprints" for the identification of species. In a monograph published by the Sahni Institute, they have revised and defined all the known species of *Glossopteris* known from 1828 to 1979. A similar revision of *Glossopteris* species from Australia is ready for publication.

## Selected Publications

1. Surange K R, A contribution to the morphology and anatomy of the Cyclanthaceae, *Trans natn Inst Sci India*, **3**(4) (1948) 159-209.
2. Surange K R, The morphology of *Botryopteris antiqua* with some observations on *Botryopteris ramosa*, *Palaeobotanist*, **1** (1952) 420-34.
3. Surange K R, The morphology of *Stauropteris burntislandica* P. Bertrand and its megasporangium *Bensonites fusiformis* R. Scott, *Phil Trans R Soc*, **237** (642) (1952) 73-91.
4. Surange K R, *Botryopteris elliptica* sp nov from the Upper Carboniferous of England, *Palaeobotanist*, **3** (1954) 79-86.
5. Surange K R, *Indian Fossil Pteridophytes* (CSIR, New Delhi) 1966, 204.
6. Surange K R & Maheshwari H K, Some male and female fructifications of Glossopteridales from India, *Palaeontographica*, **129B**(4-6) (1970) 178-92.
7. Surange K R & Chandra S, *Dictyopteridium sporiferum*, Feist-Female cone from the Lower Gondwana of India, *Palaeobotanist*, **20**(1) (1973) 127-36.
8. Surange K R & Chandra S, *Denkania indica* gen et sp nov—A glossopteridean fructification from the Lower Gondwana of India, *Palaeobotanist*, **20**(2) (1973) 264-68.
9. Surange K R & Chandra S, *Partha*—A new type of fructification from the Lower Gondwana of India, *Palaeobotanist*, **20**(3) (1973) 356-60.
10. Surange K R & Chandra S, Morphology of the gymnospermous fructifications of the *Glossopteris* flora and their relationship, *Palaeontographica*, **149B**(5-6) (1975) 153-80.
11. Chandra S & Surange K R, Cuticular studies of the reproductive organs of *Glossopteris*: Part I—*Dictyopteridium feistmanteli* sp nov attached on *Glossopteris tenuinervis*, *Palaeontographica*, **156**(4-6) (1976) 87-102.
12. Chandra S & Surange K R, Cuticular studies of the reproductive organs of *Glossopteris*: Part II—*Cistella* type fructifications, *Plumsteadistrobus ellipticus* gen et sp nov attached on *Glossopteris tenuioides*, *Palaeobotanist*, **23**(3) (1976) 161-75.
13. Chandra S & Surange K R, Cuticular studies of the reproductive organs of *Glossopteris*: Part III—Two female fructifications, *Jambadostrobus* and *Venustostrobus* borne on *Glossopteris* leaves, *Palaeontographica*, **164** (4-6) (1977) 127-52.



14. Chandra S & Surange K R, Cuticular studies of the reproductive organs of *Glossopteris*: Part IV—*Venustrostrobus indicus* sp nov, *Palaeobotanist*, **24**(3) (1977) 149-60.
15. Chandra S & Surange K R, Revision of the Indian species of *Glossopteris*, *Monograph No 2* (Bribal Sahni Institute of Palaeobotany, Lucknow) 1979, 301.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

## R N Tandon

Work on fungal taxonomy was begun by Tandon in 1927; fungal flora of different regions of the country were covered. An exhaustive list of fungi found in plains and hilly regions of Uttar Pradesh and neighbouring states was prepared. More than 60 new fungal reports were made from India during 1928-1980. Many of these were new taxa.

Studies on fungal diseases, especially those responsible for causing damage to crop plants and fruit trees, have yielded interesting results. Main emphasis was on the post-harvest diseases. The effects of many physical and environmental factors responsible for rapid deterioration of fruits and vegetables under different field and storage conditions were evaluated and control measures were suggested. Extensive studies on the storage diseases of a number of important fruits like mango, banana, guava, apple, pear, litchi, papaya, tomato and sapota, etc. were conducted and the role of different plant pathogens responsible for spoilage was assessed. The nature and potentiality of different cell wall degrading enzymes, with special reference to cellulolytic and pectolytic enzymes, were examined. It was found that temperature and moisture levels of the storage structures determined largely their activity. Detailed studies on the extracellular enzymes secreted by the species of *Fusarium*, *Alternaria*,

*Helminthosporium*, *Curvularia* and *Pestalotiopsis* were undertaken.

Studies relating to fungal physiology and nutrition were conducted on different strains and species of about 45 pathogenic genera of fungi responsible for storage diseases of fruits and vegetables and the leaf spot diseases. These studies not only helped in giving a better understanding of the life processes of the organisms, but also established that it was not proper to accept various types of classifications based only on nutritional studies of fungi, because different species of the same genus and even different isolates of the same species show marked differences in their nutritional requirements. Loss of fertility of fungal cultures was studied and it was found that different isolates and even different ascospores of the same ascus differ in fertility and the subsequent behaviour depended on the inoculum taken during subculturing. The role of synthetic transient oligosaccharides in carbohydrate metabolism in fungi was examined. It was found that the synthesis of such oligosaccharides *in vivo* was of great advantage to the pathogen in the conservation and proper utilization of the carbohydrates present in the host tissues.

Post-infection changes brought about in fruits and vegetables under pathogenesis were examined. Qualitative and

---

Formerly, Professor & Head, Department of  
Residence : C1/1 Ansari Nagar, New Delhi-110029.

Botany, Allahabad University, Allahabad-211002;



quantitative losses in the levels of carbohydrates, proteins and vitamin C contents were also assessed.

It was found that the pathogens not only modified greatly the organic acid contents of their respective hosts but also brought about drastic changes in carbohydrates of host tissues, thereby setting on the rot. The infection induced rapid decline in ascorbic acid contents of different fruits like guava, mango, papaya, bittergourd, chilli, etc. resulting in lowering of their food value.

Control measures against a number of diseases have been recommended.

Tandon has published more than 250 papers in well known Indian and foreign journals.

### Selected Publications

1. Tandon R N, *Physiological studies on some pathogenic fungi—A monograph* (Scientific Research Committee, Uttar Pradesh) 1961, pp. 80.
2. Tandon R N & Chandra S, Supplement to list of Indian fungi (1957-62), *Allahabad University Studies*, 1964.
3. Tandon R N, Comparative studies on carbon nutrition of some isolates of *Colletotrichum gloeosporoides*, *Presidential Address, Nat Acad Sci (Biological Section)*, 1965.
4. Tandon R N, Observation on storage diseases of certain fruits, *Presidential Address, Indian Phytopathological Society*, 1967.
5. Tandon R N, Certain aspects of fungal nutrition, *Presidential Address, Indian Science Congress Association (Botany Section)*, Hyderabad, 1967.
6. Tandon R N, *Mucorales of India—A monograph* (Indian Council of Agricultural Research, New Delhi) 1969.
7. Tandon R N, Certain problems of post-harvest diseases of fruits and vegetables. VII, *Mundkur Memorial Lecture, Indian Phytopath* (1970).
8. Tandon R N & Singh R H, Post-infection changes caused by *Aspergillus niger* in amino acids and organic acids of pomegranate, mango and guava fruits, *Phyton*, **27** (1970) 101-5.
9. Tandon R N & Mitra S K, Nitrogen requirements of certain isolates of *Alternaria tenuis* Auct, *Mycopath Mycol Applic*, **42** (1970) 1-2.
10. Tandon R N, Nitrogen requirements of three species of *Pestalotia*, *Mycopath Mycol Appl*, **42** (1970) 9-16.
11. Tandon R N & Kapoor I J, Biochemical and pathological studies on *Macrophoma* rot of guava (*Psidium guajava* L), *Phytopath Z*, **70** (1971) 137-44.
12. Tandon R N, Studies on post-harvest diseases of fruits and vegetables, *70th Birthday celebration of Prof S N Das Gupta Memorial Volume, Curr Trends Pl Path* (1974) 209-20.
13. Jamaluddin, Tandon M P & Tandon R N, Influence of *Myrothecium roridum* infection on ascorbic acid content of tomato fruits, *Indian Phytopath*, **27** (1974) 429-30.
14. Jamaluddin, Tandon M P & Tandon R N, Post-harvest decay of fruits of tomato caused by *Cylindrocladium scoparium*, *Indian Phytopath*, **27** (1974) 489-92.
15. Tandon R N, Utilization of polysaccharides of four pathogenic fungi causing fruit rot diseases, *Prof M R Dhar volume, National Acad Sciences, Allahabad*, 1973.
16. Tandon R N, Physiology of reproduction in fungi, *Presidential Address, Nat Acad Sciences, Allahabad*, 1976.
17. Tandon R N & Kapoor I J, Systemic fungicides on the control of some fruit diseases, *Prof P Pariya's 85th Birthday Celebration Volume, Frontiers of Plant Sciences, Utkal University, Bhubaneshwar (India)* (1977) 232-42.

## M J Thirumalachar

Thirumalachar carried out his research work during different periods in Botany Department, Central College, Bangalore; Mycology Laboratory, Department of Agriculture, Bangalore; Banaras Hindu University, Varanasi; Central Potato Research Institute, Patna; Hindustan Antibiotics, Pune; University of Wisconsin, Madison, Wis.; Istituto Superiore di Sanita, Rome; Seed Pathology Research Institute, Copenhagen; Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minn.; DOW Chemical Laboratories, Walnut Creek, California; and Jeersannidhi Anderson Research Institute, Walnut Creek, California.

### Mycology and Plant Pathology

Thirumalachar had the initial training under Dr M J Narasimhan, Sr Plant Pathologist and Director of Agriculture, Mysore State, and Dr B B Mundkur, Indian Agricultural Research Institute, New Delhi. Work on morphology, cytology and parasitism of many rust fungi was done, reporting some unique and outstanding features, such as development of aeciospores and teliospores within spermogonia in *Uromyces hobsoni* and *Zaghouania oleae*. Heteroecious life-cycles of many of the rusts were established for the first time, as in the bamboo rust *Dasturella divina*, *Puccinia cacao*, *P. versicolor*, *P. agrophila*, *Uromyces clygnii*, *Uromyces setariae-italicae* (all in

joint authorship with M. J. Narasimhan Sr) and *Uromyces commelinae*.

The genera of rusts were covered in monographs published in three parts in 1949-1950 for the first time in joint authorship with B B Mundkur, and this formed the basis for later monographs by other urediniologists. *Didymopsorella*, *Kernella* and *Hiratsukamyces* were described as new genera and several new species of rusts were described. The Ustilaginales (smuts) of India were covered in a monograph published in joint authorship with Mundkur as a special publication of Commonwealth Mycological Institute, Kew, UK. Morphology, cytology and life-cycles of many smut fungi were described, including the new genera established under the names *Mundkurella*, *Narasimhanina*, *Jamesdicksonia*, *Franzpetrakia*, *Georgefischeri* *Zundelula*, *Angiosorus* (smut on potato tubers), and *Tolyposporidium* (formerly called *Tolyposporium*).

In joint authorship with Narasimhan (Sr), the downy mildews of cereals and grasses were investigated, and the genus *Sclerophthora*, intermediate between *Sclerospora* and *Phytophthora*, was established. *Peronosclerospora dichanthicola*, *P. westonii* and *P. iseilemat* were described as new species. Morphological, cultural and systematic studies on the Indian species of



*Cephalosporium* (*Acremonium*?), *Conidiobolus*, *Elsinoe* and *Sphaceloma*, were made. Work on the *Cercosporae* of India, *Ephelis* and *Balansiae* of India, species of *Physoderma* in India was published in several parts. The new myriangiaceous genera *Bitancourtia*, and *Annajenkinsia*, and the mucoraceous genus *Thermomucor* were reported. A large number of Meliolinae, including many new species, were described in joint authorship with C G Hansford.

Advanced training in plant pathology leading to PhD degree at the University of Wisconsin was under Dr James G Dickson and Dr J C Walker, and post-doctoral work was done with Dr Charles Chupp (Cornell University, Ithaca, N. Y.), Dr F D Kern (Penn State University), Dr Anna E Jenkins (USDA), and Dr G B Cummins (Purdue University).

As Chief Plant Pathologist at the Central Potato Research Institute, Patna, Thirumalachar developed control measures for charcoal rot of potato (*Macrophomina phaseolina* and *Botryodiplodia solani-tuberosi*) by cultural practices, and by incorporating immunity from *Solanum chacoense* of the Commeroniana potato group. Potato leaf-roll virus control by summer storage of diseased tubers in the Indian plains as a result of thermal inactivation was first demonstrated and later confirmed by the laboratory findings of Kassanis at Rothamsted, UK and field studies in New Zealand.

Biological control of plant pathogens using microbial antagonists was developed on an industrial scale, specially for root rot inciting organisms bearing sclerotia. A new strain of *Bacillus subtilis*, which as seed

treatment controlled charcoal rot of potatoes, and *Erwinia* soft rot as tuber treatment, was later confirmed as the most promising organism ever for the control of bean rust *Uromyces phaseoli* as foliar spray by workers in Beltsville, USDA [Phytopathology, 73 (1983) 1148-52]. Greening of potato tubers immediately after harvest and seed tubers stored in this form became immune to charcoal rot during summer storage, dry rot, *Bacillus polymyxa* rot and damage by rodents due to a polypeptide compound formed during greening. Valuable seed tubers can be saved by this greening process which avoids the need for refrigeration in Indian plains.

New chemotherapeutants were developed for controlling plant diseases in India and abroad. Aureofungin, an aromatic heptaene, was developed, which is being manufactured by Hindustan Antibiotics, Pimpri, Pune. Its high antifungal activity is indicated by six grams of the substance being capable of controlling disease in one acre field crop as spray. A new antibiotic combination, Streptocycline, also marketed by Hindustan Antibiotics, controls plant bacterial diseases. A new chemical chemotherapeutant, KT-19827, which is a soluble form of copper, with no phytotoxicity but high fungicidal-bactericidal activity, and more than all systemic in plants, has been developed. It has been registered under EPA in USA under the name Phyton-27 and is commercially produced and marketed for control of Dutch elm and oak wilt diseases, and as seed treatment of various crop plants in the United States. A new chemotherapeutant for control of mycoplasma and fastidious xylem and phloem inhabiting bacteria has been

developed and used in USA for control of many important plant diseases, such as citrus greening and die-back, almond leaf scorch, pear decline, plum leaf scald, cherry buck. skin and others. In 1984 in limited testing on citrus greening and die-back carried out in IARI, New Delhi, good remission of greening symptoms was obtained. A few sandal spike affected trees treated with this chemotherapeutant in Mysore recovered completely. Tetracyclines give only temporary remission, and the disease recurs.

A medical mycology school developed at the Hindustan Antibiotics with joint research collaboration of B J Medical College, Pune, Aundh Chest Hospital, Pune, developed methods for handling the organisms and evaluation of chemotherapeutants for disease control. Personnel trained under the project hold important positions in medical mycology field in India and abroad. Hamycin therapy for monilial and trichomonal vaginitis, trichophytosis, and other dermatophytosis, and as oral treatment for the control of secondary opportunistic fungi, pulmonary, intestinal and ocular, following broad spectrum antibiotics was established. The elimination of resistance factor (plasmids?) in *Mycobacterium tuberculosis* resistant to streptomycin, INH and PAS, when given with oral Hamycin, has great therapeutic promise. New organisms inciting madura foot, *Cephalosporium madurae*, *C. infestans*, were described, and the role of oral Hamycin in regression of disease symptoms was shown.

### Cell Research and Cell Metabolites

During his PhD work at the University of Wisconsin, Madison, Thirumalachar was trained under Prof M P Backus, who

developed Wisconsin strains of *Penicillium chrysogenum* used world over for commercial penicillin production. In 1953-54, he was trained under Sir E B Chain, Nobel Laureate, discoverer of penicillin. During this period, the new streptomycete genus *Chainia* was discovered. He worked for short periods on research problems on antibiotics with Dr Selman A Waksman, Academician N A Krassilnikov, USSR and Dr Ernst Gaumann, E T H, Zurich.

As Chief Mycologist, and later Head of the Research Centre, Hindustan Antibiotics, Poona, Thirumalachar developed high yielding strains of commercially produced antibiotics, new testing and screening methods, and several antifungal, antibacterial, antiparasitic and anti-tumour antibiotics. Some of these are briefly mentioned below.

(1) Hamycin, a new heptaene antibiotic with high antifungal activity, is being used in India therapeutically for monilial and trichomonal vaginitis, and work at NIH, Bethesda, MD, USA showed its remission activity against north American blastomycosis, histoplasmosis and cryptococcosis. Hamycin has been listed in most of the medical textbooks and in Merck Index.

(2) Dermostatin, an unusual type of hexaene with lactone with high activity against dermatophytosis was discovered. Complete structures of Hamycin and Dermostatin were published by Dr K L Rinehart of University of Illinois, Urbana. Eli Lilly group tested Dermostatin by IP route in mice and reported that its activity was equal to that of Amphotericin-B. Dermostatin is listed in Merck Index.



(3) Discovered and developed Streptorubrin A and B which were shown to have high cytostatic activity under the National Cancer Research, Bethesda programme. Their physico-chemical properties have been included in the Index of Antibiotics from Actinomycetales published by Dr Hamao Umezawa.

(4) Discovered and developed Aureofungin, a new antifungal aromatic heptaene, whose chemical structure was described by Dr Rinehart. It is being manufactured by Hindustan Antibiotics as a fungicidal pesticide.

(5) Described and developed the new antibiotics, Neopentaene, HA-135, HA-145 and Chainin, which are all pentaenes, and Hexin, a hexaene antibiotic.

(6) Described the new tetraenes, Tetraenin A and Tetraenin-B.

(7) Developed the antitumour antibiotic necrocitin from strains of *Myrothecium roridum*, which is structurally different from the Mucanomyces, and roridins. Its activity against plant tumours was established. At concentration of 0.01 µg/ml, it is highly molluscidal.

(8) Discovered and developed the new polypeptide antibiotic Antiamoebin, whose complete structure was published by Dr K L Rinehart. Antiamoebin is a potent antiprotozoal-anthelmintic antibiotic and at present is the most potent antibiotic against trypanosomiasis.

(9) Discovered and developed the new antibiotic Antiprotozoin, which is both antifungal and antiprotozoal and has a phosphoinositide group in its molecule.

(10) Discovered the new antibiotic Fucothricin among the streptothricin group with a fucose moiety. It is the least

toxic among the streptothricins. It has strong activity against *Mycobacterium*.

(11) Developed TC-1474, a new antimycoplasmal agent for the treatment and control of *Mycoplasma gallisepticum* and *Salmonella gallinarum* in chicks, when used as a feed additive.

Thirumalachar described *Cephalosporium chrysogenum* Thirum & Suka isolated from a soil sample in Pune, which is the strain commercially used for the commercial production of Cephalosporin-C.

A new process was developed for the production of human insulin by genetically transforming fungal cells (*Pseudosaccharomycete* TC-1176, ATCC 20,477) using a nonrecombinant DNA technique, the functional genome used for the transformation coming from human beta-cells grown in secondary serial cultures using a new process. The transformed fungal cells are then grown in mass submerged cultures as in antibiotic fermentation type, and the human insulin extracted and identified as whole insulin molecule by radio-immune assay. This was in 1978 long before any yeast or much less pseudoyeast was considered for genetic transformation studies, and only *E. coli* were being experimented with. The insulin produced by Centatech-Eli Lilly process gives a and b chains of insulin as separate units and these then are chemically bonded, unlike the present process (U.S. Pat 4,082,613, 4 April 1978, M J Thirumalachar, M J Narasimhan Jr and John A Anderson, assigned to the Regents of the University of Minnesota). Financial considerations have delayed exploitation of the process commercially.

In the field of environmental microbiology, the oil spills polluting the ocean have been a major problem affecting sea life and polluting the coastal areas. Numerous bacterial organisms, some of them genetically engineered to fix atmospheric nitrogen for survival in ocean environment, have failed to yield the desired results. A new species of fungus, *Geotrichum marinum* Thirum., isolated and developed from oil spills in the Pacific Ocean, has the ability to degrade petroleum materials as in oil spills, thereby bringing about effective degradation (U.S. Pat 4,415,661, 15 November 1983).

Oil tankers after discharging the cargo in ports have large quantities of residues left on the ship surface and holding tanks; the problem of cleaning up has never been solved satisfactorily. A marine strain of *Actinomucor elegans* produces an enzyme which even in crude form cleans up the petroleum oils on the ship surface (U.S.

Pat 4,415,662, 15 November 1983). Large-scale handling of both the organisms for cleaning up crude petroleum oils is underway.

A new micro-organism, which fixes atmospheric nitrogen in the rhizosphere of corn plants and improves the nutritive value of corn by storing more lipids in the grains has been tested in a 2-acre corn plot in commercial planting. Using all agricultural practices in mechanically planted and harvested crop, half fertilized field where the seed treatment with the organism was done, gave the same yield as the fully fertilized field. Large-scale trials in several of the corn growing states in USA are planned. The organism is able to give good growth on nitrogen-free media in artificial cultures, and is very different from the bacterial species belonging to *Azotobacter* and *Azospirillum* now being studied in various laboratories of the world.



## B S Trivedi

After passing the MSc examination from Lucknow University in 1943, Trivedi started research under late Prof Birbal Sahni, FRS and worked as his research assistant for some time, subsequently becoming Lecturer, Reader and Professor and Head of the Department of Botany, Lucknow University.

During the last 42 years, he has carried out intensive and extensive work on fossil plants, mainly of Tertiary age from India and Malaya. His first paper appeared in *Nature* in 1944, in collaboration with late Prof. Sahni, on the age of the Saline series of Salt range; the question of age was highly controversial at that time. This paper, as also several subsequent publications by the Lucknow School, demonstrated conclusively that the problems of age of beds or strata of doubtful age could be solved by studying their microfossil content. With this paper, Trivedi earned recognition in the palaeobotanical world. Later, he published some important papers on the megaspores of lower Gondwana of Singrauli coalfield. These were important contributions in the field of micropalaeobotany.

After 1960, Trivedi paid attention to the excellently preserved plants of the Deccan Intertrappean beds. He has carried out extensive work on the Deccan Intertrappean Flora by describing and

reporting 40 taxa of angiosperms from these beds, many of them being new to science. He has reported algae, fungi, Pteridophytes, and Angiosperms, both monocotyledons and dicotyledons, from the Intertrappean beds, which are exposed at Nagpur, Chhindwara and Mandla districts of Madhya Pradesh. Among the Pteridophytes, a new species of *Azolla*, a water fern, has been described in detail; comparisons of all the known fossil species of *Azolla* have been made. Among the Angiosperms, *Nymphaeocaulon* (Nymphaeaceae), *Cannaites* (Cannaceae), *Heliconiaites* (Heliconiaceae) and *Cyclanthodendron* (Cyclanthaceae) have been reported. In addition to these, numerous other monocot and dicot woods have been described. About 10 new fossil dicot wood genera have been created by him. On the basis of varied plant fossil assemblage from the Deccan Intertrappean beds, palaeoecology of the different localities has also been studied.—Other Tertiary horizons which have been worked out by Trivedi are the Mio-Pliocene beds of Kalagarh, UP. About 30 new dicot taxa have been described from this locality. The fossil dicot woods throw light on the distribution of these taxa during Mio-Pliocene time.

Tertiary plant remains of Malaya were studied by Trivedi, when he visited Malaya in 1953 and collected a large number of

---

Formerly, Professor & Head, Department of Botany, University of Lucknow, Lucknow-225007; Residence : B-8, Sector A, Mahanagar, Lucknow-225006.

coal and shale samples. Previously, these rocks were supposed to be unfossiliferous. Trivedi demonstrated that the Malayan coal was highly fossiliferous. He reported about 60 new microfossils, which include fungi, pteridophytic spores and pollen grains, tracheids and cuticles.

He also published a paper on the Jurassic plant remains from Burma.

Trivedi has made valuable contributions on fern gametophytes. He studied the effects of strong magnets and various chemicals on the gametophytes of *Lygodium* and *Pteris*. His TEM studies have shown as to how different cell organelles disorganize due to the forces generated by strong magnets. The effect of gamma ray radiation on the structure of fern gametophytes has been studied by LM and TEM.

Epidermal structures, including the ontogeny of stomata of various families of angiosperms and of some taxa of uncertain systematic position and polyploids have been studied in detail. Epidermal structures of about 500 taxa belonging to Apocynaceae, Asclepiadaceae, Gentianaceae and Oleaceae (dicots); Palmae, Araceae and Dioscoriaceae (monocots) have been described. He has made a key for the identification of taxa using epidermal characters.

Trivedi has made notable contributions to the seed coat structure of some economically important families. Members of the family Leguminosae have been studied in depth. SEM studies on the spermoderm pattern of a large number of seeds of the tribes Genisteae, Trifolieae and Phaseolae of Papilionatae and members of Mimosoideae have been described. SEM studies have shown that

the seed coat has a very definite and characteristic structure, which can help in the identification of seeds of the various tribes and genera. Trivedi has made valuable and significant contributions to seed coat morphology.

### Selected Publications

1. Sahni B & Trivedi B S, Age of Saline series in the salt range of the Punjab, *Nature, Lond*, **184** (1944) 54.
2. Trivedi B S, Megaspores and other plant remains from Lower 'Gondwana of Singrauli Coal field, district Mirzapur, U.P., *J Indian bot Soc*, **32**(1) (1953) 70-85.
3. Trivedi B S & Verma C L, The structure of Pseudostem and root of *Cannaites intertrappea* gen et sp nov from the Deccan Intertrappean beds of MP, India, *Palaeontographica*, **B132** (1971) 175-85.
4. Trivedi B S & Verma C L, Contributions to the knowledge of *Azolla indica* sp nov from the Deccan Intertrappean series, MP, India, *Palaeontographica*, **B136** (1971) 71-82.
5. Trivedi B S & Verma C L, Occurrence of *Heliconiaites mohgaonensis* gen et sp nov from the early Eocene of Deccan Intertrappean series, MP, India, *Palaeontographica*, **B139** (1972) 73-83.
6. Trivedi B S & Ambawani K, On the structure of *Nymphaeocaulon intertrappea* gen et sp nov from the Deccan Intertrappean series of Mohgaon (MP), India, *Palaeontographica*, **B133** (1971) 29-36.
7. Trivedi B S & Verma C L, *Cyclanthodendron* remains from the Deccan Intertrappean beds of Madhya Pradesh, India, *Palaeobotanist*, **25** (1978) 529-42.
8. Trivedi B S & Verma C L, A new fossil fungus *Ascodesmusites malayensis* gen et sp nov from Tertiary coal of Malaya, *Geophytology*, **3**(2) (1972) 126-29.
9. Trivedi B S & Verma C L, Polynological studies of coal of Malaya with reference to its age, *J Indian bot Soc*, **61**(4) (1982) 432-43.
10. Trivedi B S & Kher U, Effect of strong magnets on the ultrastructure of Plastids of *Lygodium flexuosum* (L) SW, *Phytomorphology*, **26**(2) (1977) 222-24.



11. Trivedi B S & Kher U, Ultrastructural study on the effect of colchicine on the gametophyte of fern *Lygodium flexuosum* (L) SW, *Phytomorphology*, **27**(4) (1978) 337-42.
12. Trivedi B S & Ahuja M, Two new Dipterocarpaceous woods from the middle Siwalik of Kalagarh, Bijnor District, India, *Palaeobotanist*, **26**(3) (1980) 314-21.
13. Trivedi B S & Ahuja M, *Pentacmoxylon ornatum* gen et sp nov from the Siwaliks of Kalagarh, *Curr Sci*, **48**(14) (1979) 646-47.
14. Trivedi B S & Upadhyay N, Epidermal structure in Palmae, *Biol Mem*, **4**(1,2) (1979) 83-117.
15. Trivedi B S & Upadhyay N, Cuticular studies of Asclepiadaceae, *J Indian bot Soc*, **63**(2) (1984) 129-47.
16. Trivedi B S & Bagchi G D, Scanning electron microscopic studies on the spermoderm of some mimosoideae (Leguminosae), *Phytomorphology*, **29** (1979) 211-18.
17. Trivedi B S & Bagchi G D, Scanning electron microscope studies on the spermoderm structure of tribes Genisteae, Trifolieae and Phaseolae (Papilionatae—Leguminosae), *Phytomorphology*, **32** (1982) 138-45.
18. Trivedi B S, Palms—The big game of plants, *Geophytology*, **12**(2) (1982) 137-43.
19. Trivedi B S & Singh D K, *Introduction to Gymnosperms* (Shashi Dhar Malviya Prakashan, Lucknow) 1966, pp 117.
20. Trivedi B S & Sharma B B, *Introductory taxonomy (Angiosperms)* (Kitab Mahal, Allahabad) 1983, pp 336.

# G S Venkataraman

## Cyanobacterial Nitrogen Fixation

Venkataraman is a pioneer in the field of blue-green algae (Cyanobacteria), and researches in India on biological nitrogen fixation by Cyanobacteria and its utilization in agriculture owe much to him. He is the chief architect of the algal biofertilizer technology for rice and is mainly responsible for giving an effective direction to the economic utilization of the renewable algal resources for fertilizer, feed and fuel in India. He has established an active school in agricultural algology at the Indian Agricultural Research Institute (IARI), New Delhi. The 'Culture Collection of Microalgae' established by him at IARI is the biggest in this part of the world and is depended upon by research workers and institutions within and outside the country. Author of six monographs and over 200 research papers, he is the Editor of Publications (Biological Sciences) of the Indian National Science Academy, Chief Editor of Phycological Society of India and member of the Editorial Boards of many scientific bodies.

His research contributions cover a wide range of basic and applied aspects of biological nitrogen fixation and span over a period of 30 years. His major contributions relate to (i) distributional pattern, seasonal succession and strain variation, (ii) elucidation and characterization of the ecophysiological and management factors

influencing growth and nitrogen fixation, (iii) regulation of nitrogenase activity by ecological stresses, (iv) serology of blue-green algae, (v) environmental role of algae, (vi) crop-alga interaction with reference to transfer of fixed nitrogen, (vii) improving strain efficiency by selection and mutation, (viii) development of the concept of mixed inocula for field application, (ix) development of a rural-oriented algal biofertilizer technology to supplement the chemical nitrogen fertilizer, (x) energy contribution and balance through algal resources, (xi) large scale transfer of this technology to the farmers in many parts of the country, supported by national level extension and training programmes, and (xii) algal biomass production for supplementary protein. The algal technology, if used extensively, has the potential to provide 7-8 lakh tonnes of biologically fixed nitrogen to our entire rice crop ecosystem.

## Distributional Pattern and Strain Variation

His work has shown that contrary to the generally held belief, nitrogen fixing blue-green algae do not seem to be invariably present in all tropical rice soils, although recurrent combinations of algal species may occur in comparable habitats. In India, as low as 7% to as high as 86% of rice field soils harbour useful blue-green algae, in different regions. Besides, there is



a wide range of inter- and intra-generic and species variation in the nitrogen fixing potential. The kinetics of nitrogen fixation and excretion by different strains show that the important factors involved in regulating algal activities in natural ecosystems are (i) the relative efficiency of strains, (ii) rate of excretion and availability of extracellular nitrogenous substances, and (iii) root environment. His work on cyanophages has shown the importance of biological factors in determining the establishment of specific algal strains in a particular ecosystem.

### Soil Properties

The soil aggregation status improves significantly due to algal inoculation, an observation which has great agronomical significance. In problem soils, such as salt-affected ones, a perceptible reduction is brought about by algal growth in pH, electrical conductivity and exchangeable sodium status. This reclamative potential of certain blue-green algal strains has ecological and agronomical implications.

### Regulation of Nitrogenase

One of his current interests is in the regulation of cyanobacterial nitrogenase by exogenous factors, such as combined nitrogen ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ), antibiotics and chelating agents. He has isolated a number of strains whose nitrogenase shows differential response to  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . While most of the strains normally show an inhibition with  $\text{NH}_4^+$ , a few are insensitive to both  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , and a couple of strains are sensitive to  $\text{NO}_3^-$ , but not to  $\text{NH}_4^+$ . Such strains provide source material for genetic manipulation.

### Pesticide Tolerance

His approach to induce high levels of tolerance to pesticides helps to understand the interactions in complex model systems. Use of such pesticide resistant mutants will also restore an important source of biological nitrogen, particularly when herbicides applied to fields to kill the weeds also kill the algae.

While biological nitrogen fixation in agricultural ecosystems is rarely limited by a lack of nitrogen fixing microorganisms, a variety of environmental pressures may prevent them from doing what the glass-house and pot-culture studies suggest they ought to do. One should not only define these stresses, but also devise methods to overcome them. His current interest and work are in this direction.

He has also made significant contributions to the understanding of the ecology of soil microorganisms, biocoenotic interactions in soil ecosystems, genetic transfer and repair mechanism in blue-green algae, *Rhizobium* and *Azotobacter* and to the use of algal biomass as a source of high quality supplementary protein.

### Selected Publications

1. Venkataraman G S, *The cultivation of algae* (ICAR, New Delhi) 1969, pp 319.
2. Venkataraman G S, Blue-green algae production for rice, *FAO Soils Bull* No 46 (FAO, Rome), 1981, pp 102.
3. Venkataraman G S & Neelkantan S, Effect of cellular constituents of the nitrogen fixing blue-green alga *Cylindrospermum muscicola* on the root growth of rice seedlings, *J gen appl Microbiol*, **13** (1967) 53-61.
4. Lorenzen H & Venkataraman G S, Synchrony in blue-green algae, in *Methods in Cell Physiology*, Vol 5, edited by D M Prescott (Academic Press, New York) 1972, 373-83.

5. Venkataraman G S, Progress in biological nitrogen fixation, *Acta Bot Indica*, **3**(1) (1975) 23-29.
6. Sharma C R, Venkataraman G S & Nam Prakash, Cyanophage AC-1: Mode of infection, *Phykos*, **18** (1979) 57-62.
7. Venkataraman G S, Algal biofertilizer: Potential and problems, *Keynote Address, Natn Workshop on Algal Systems, Indian Soc Biotech*, 1980, 1-10.
8. Kaushik B D, Sharma C R, Venkataraman G S & Sen A N, Serologic relations among blue-green algae, *Curr Sci*, **50** (1981) 134-37.
9. Venkataraman G S, Economics and energetics of blue-green algal contribution to rice, *Curr Sci*, **50** (1981) 94-95.
10. Venkataraman G S, Blue-green algae: A possible remedy to nitrogen scarcity, *Curr Sci*, **50** (1981) 253-56.
11. Rao D L N, Venkataraman G S, Duggal S K & Eggum B O, Nutritional quality of the blue-green alga *Spirulina platensis*, *J Sci Fd Agric*, **33** (1981) 456-60.
12. Venkataraman G S, Nitrogen fixation by blue-green algae and its economic importance, *Proc 12th Int Cong Soil Sci*, New Delhi, 1982, 69-82.
13. Venkataraman G S, Some future approaches to photoautotrophic nitrogen fixation, *Proc natn Symp Biol Nitrogen Fixation*, BARC, Bombay, 1982, 17-25.
14. Venkataraman G S & Watanabe I, Biological nitrogen fixation, in *Rice research strategies for the future* (Int Rice Res Inst, Los Banos, Philippines) 1982, 317-26.
15. Kaushik B D & Venkataraman G S, Response of cyanobacterial nitrogen fixation to insecticides, *Curr Sci*, **52** (1982) 321-23.
16. Roychoudhury P, Pillai G R, Pandey S L, Krishna Murti G S R & Venkataraman G S, Effect of blue-green algae on aggregate stability and rice yield under different irrigation and nitrogen levels, *Soil & Tillage Res*, **3** (1983) 61-65.
17. Chandrasekharan P A & Venkataraman G S, Regulation of nitrogenase activity in *Anabaena variabilis*, *Proc Indian natn Sci Acad*, **51B** (1985) 78-84.





**Sectional Committee—VII : Animal Sciences**  
**(Structural, Developmental, Functional, Genetical,**  
**Ecological, Behavioural, Taxonomical and**  
**Evolutionary Aspects)**





## T N Ananthakrishnan

The principal areas of investigation during the period 1946-86 were confined to (a) Taxonomy, bioecology of Thysanoptera, (b) Cecidology, (c) Insect-crop-weed interactions in relation to host specificity, and (d) Pollination biology. The first phase of my studies (1946-60) essentially involved extensive work on the bio-taxonomy of thrips, an economically important group of insects; around 50 new genera and over 200 new species of thrips were discovered during this period. The role of thrips in agriculture, horticulture and forestry was assessed on the basis of bioecological studies on several species. The second phase (1960-75) involved in-depth studies on the mycophagous Tubulifera inhabiting the saprophytic fungal zone, resulting in the discovery of profound sex-limited intraspecific diversity, leading to oedymmerism and gynaecoidism in males. Of particular significance is the fact that new features tend to express themselves at the normal-oedymere transitions of a species followed by their maximum expression in the extreme oedymeres. Correspondingly significant decrease in the degree of development of secondary sexual characters was evident from the normal to gynaecoids. Diverse patterns of intraspecific sex-limited polymorphism were identified, the basic ones being designated simple, monophasic as well as multiple, polyphasic. Multiple effects of

oedymmerism were recognized in several species and allomorphic indices were formulated.

Intensive studies on cecidological aspects of thrips resulted in the discovery of several genera and species of gall thrips as well as of the range of gall diversity. More than 120 species of gall thrips were recorded, including nearly 25 new genera and 70 new species. Besides the study of ecological aspects of galls involving the nature of gall association, polymorphism and population trends, an exhaustive key to the gall thrips genera of the world was prepared. Histopathological aspects of different gall types were also highlighted. Thrips-weed-crop interactions were examined in detail to study the role of weeds as reservoirs of thrips pests in cultivated fields.

During the third phase (1975-85), emphasis was laid on the behavioural trends in feeding and reproduction in relation to the degree of host specificity of some species of Orthoptera, Hemiptera and Thysanoptera, involving several biochemical parameters. Studies on the bioecology of mycophagous thrips involved an analysis of fungal food and its impact on the reproductive biology of several mycetophagous and sporophagous thrips, as well as the vector relationships of some of the sporophagous species in



the spread of fungal diseases in forests and plantation crops. The pollination potential of thrips was examined, with particular reference to the natural order Asteraceae in an effort to highlight thrips-weed-crop interactions.

### Future Directions

Electroantennogram studies to determine the role of sensillae and concerned receptor systems in phytophagous insects in relation to host specificity studies are in progress. Further investigations on the biochemical aspects of host specificity relating to the specific chemical compounds of the host plants as well as the biochemistry of thrips galls, along with aspects of bioenergetics in terms of energy expended in the choice of, and development on, different food plants are also being contemplated.

### Selected Publications

1. Ananthakrishnan T N, Indian Thysanoptera, *CSIR Zoological Monographs*, **1** (1967) pp. 171.
2. Ananthakrishnan T N, Thrips in agriculture, horticulture and forestry—Diagnosis, bionomics and control, *J scient ind Res*, **30** (1971) 113-46.
3. Ananthakrishnan T N, *Thrips, biology and control* (Macmillan India), 1973.
4. Ananthakrishnan T N, The distribution and host range of predatory thrips, *Indian J Pl Prot* No **4**(1) (1974) 67-78.
5. Ananthakrishnan T N, *Insects and host specificity* (Macmillan India), 1977.
6. Ananthakrishnan T N, Thrips galls and gall thrips, *Zoological Survey of India, Technical Monograph*, **1** (1978) pp 69.
7. Ananthakrishnan T N, Biosystematics of Thysanoptera, *A Rev Entomol*, **24** (1979) 159-83.
8. Ananthakrishnan T N, On some aspects of thrips galls, *Bull Soc biol Fr*, **127** (1980) 31-34.
9. Ananthakrishnan T N, Thrips, in *Vectors of plant pathogens* (Academic Press, Inc), 1980, 145-64.
10. Ananthakrishnan T N, Thrips-plant gall association with special reference to patterns of gall diversity in relation to varying thrips populations, *Proc Indian natn Sci Acad*, **B47**(1) (1980) 41-46.
11. Ananthakrishnan T N, Thrips and pollination biology, *Curr Sci*, **51**(4) (1982) 168-72.
12. Ananthakrishnan T N & Suresh G, Patterns of fungal resource utilization and feeding range in some mycophagous Tubulifera, *Proc Indian Acad Sci*, **B92** (1983) 285-91.
13. Ananthakrishnan T N, *Bioecology of thrips* (Oak Park, Michigan), 1984, pp. 233.
14. *Biology of gall insects*, edited by T N Ananthakrishnan (Oxford & IBH, New Delhi) 1984.
15. Ananthakrishnan T N & Sen S, Taxonomy of Indian Thysanoptera, *Zoological Survey of India, Handbook Series 1*, 1980, pp 234.

## N Balakrishnan Nair

Among the many questions of keen scientific interest that are encompassed by the broad field of aquatic biology, none had greater economic significance than that of biological deterioration. There is no branch of human activity, including industry, which is not adversely affected by biodeterioration. Its effects are far-reaching and of incalculable cost to every national economy in terms of attack on metals, plastics, cellulose, wood and apparatus of all kinds. The extensive damages caused by marine organisms that burrow or bore into underwater wooden structures and wooden crafts and the deleterious effects of animals, such as barnacles, and bryozoans that foul ship's hulls and submarine equipment are well known. These organisms hamper man's maritime activities, causing huge financial losses. Knowledge of these organisms has, therefore, considerable economic importance. Ecology of littoral benthos in general and of marine fouling and wood-destroying organisms in particular has been one of the major areas of investigation during the past 30 years, primarily in the Indian Ocean<sup>1</sup>, in the North Atlantic, North and Berents Seas and in the Arctic Ocean<sup>2</sup>, and more recently in the South Atlantic and a great volume of important work concerning the taxonomy, biology and ecology of these organisms has been published<sup>3</sup>.

In India, the attack on timber is the concerted and continuous effort of a heterogeneous assemblage consisting of at least 55 different species of crustaceans and molluscs; in addition, there is microbial cellulolytic degradation by bacteria and fungi<sup>4</sup>. These are engaged in a relentless destruction of valuable timber belonging to the poorer sections of the community, namely the fishermen, thereby reducing its service life in the sea and brackish water and even in fresh water. Enormous loss to property is caused by these pests. Actual assessment of damage is difficult, but the fishing industry alone suffers an annual loss of more than Rs 10 million. The nature of the organisms, their incidence and relative abundance along the extensive coastline of India and in the insular situations of Lakshadweep, the Andaman and Nicobar Islands have been noted carefully and the factor or factors responsible for their survival, growth and distribution have been examined. The role of mangroves in the maintenance of a perennial source of borers has also been recorded. Information on the nature of vertical distribution of wood borers has been collected, since the degree of deterioration at different levels along a pile is based on the intensity of settlement and growth at these levels<sup>5</sup>. The time of settlement was studied carefully, since it is of special interest apart from its biological

---

Chairman, State Committee on Science, Technology & Environment and Ex-Officio Secretary to Government of Kerala, Near Press Club, Trivandrum-695001; *Residence* - 'Swathi', Residency Road, Thycaud, Trivandrum-695014.



importance. It is during this period that the infestive free-swimming larvae come into contact with fresh surfaces and experience the effect of preservatives used on them. Precise knowledge of the times of settlement of the different species in a locality is of importance in connection with such operations as replacements, dry-docking, repainting of boats, pile driving, etc. Biologically, the period and extent of settlement are significant, since they are reflections of the breeding season and a reliable index of the breeding success<sup>6</sup>.

Based on a detailed study of the frequency of occurrence of veliger larvae in the plankton, the presence of post-settled stages on test panels and the gonado-somatic index of adults, the exact breeding periods of several species has been delineated<sup>7,8</sup>.

Detailed embryology and larval ecology of the tropical shipworm *Bankia indica* (*Bankia carinata*) have been worked out step by step and stage by stage; this contribution represents the most complete and comprehensive study of the development of a shipworm so far<sup>9</sup>. It has been shown that the events leading to settlement and growth are dependent on the interplay of a large number of factors, including the physiological characteristics of the species involved, their geographical distribution, local variations in the character of temperature and salinity changes, and the seasonal influence of other less obvious aspects of the environment<sup>3</sup>. Growth rates were checked for the reason that they are directly related to damage done to timber; each shipworm destroys a column of wood of the same dimension as its largest size<sup>10</sup>.

The various environmental factors which affect the natural populations of

shipworms, such as temperature, salinity, oxygen tension, turbidity, pollutants, the presence and intensity of fouling organisms, nature of the wood depending on the species of timber, its softness and orientation of the grain, length of exposure of the sample in water, nature of preservatives used, location of the sample in relation to tidal changes, orientation in relation to depth, mechanical effects, interspecific and intraspecific relationships have also been analysed in detail<sup>2,4,11,12</sup>.

The functional morphology of several species of these highly specialized bivalves has been studied and a clear picture of the nature of specialization has emerged<sup>3</sup>. The sequence of sexual phases has been elucidated clearly<sup>13</sup> and all aspects of the breeding biology of three genera, *Teredo*, *Bankia* and *Nausitora*, have been described under tropical conditions of Indian and Atlantic Oceans<sup>8</sup>.

The food and feeding habits of shipworms have been investigated thoroughly with a view to finding out whether the cellulases and other enzymes present in the digestive tract are produced by the animal itself or by commensal bacteria. The nature and action of the various digestive enzymes have been worked out in great detail<sup>14</sup> and a clear picture of the physiology of digestion in these bivalves has emerged, including the path of enzymic hydrolysis of cellulose<sup>15</sup>.

Nair's work<sup>1,2,11</sup> on the ecology of these organisms has been considered as the foundation for further work on these groups in Scandinavia and S. America.

The characteristics of penetration of substrates by representative littoral bivalves have been elucidated<sup>16</sup>. The exact mechanism involved in the drilling process,

the role of the shell-valves, the adductor muscle, the production and maintenance of turgor within the mantle cavity and the fluid dynamics have all been explained. The adaptational sequence involved during the evolution of the rock- and wood-boring habits of the Adesmacea has been described<sup>17</sup>.

Nair's contributions to aquatic ecology fall in the areas of ecology of the mesopsammon (20 papers), reproductive biology (35 papers), functional morphology (15 papers), fishery biology (65 papers), fish pathology (35 papers), environmental pollution (45 papers), ecology of estuaries (45 papers) and some miscellaneous aspects (30 papers). He has also published four books.

### Selected Publications

1. Nair N B, Shipworms of Venezuela, *Bol Inst Oceanogr Univ Oriente*, **14** (1975) 129-46.
2. Nair N, Balakrishnan, Ecology of marine fouling and wood-boring organisms of Norway, *Sarsia*, **8** (1962) 1-88.
3. Nair N B & Saraswathy M, The biology of wood-boring teredinid molluscs, *Adv mar Biol*, **9** (1971) 335-509.
4. Nair N B, The problem of marine timber destroying organisms along the Indian coasts, *Proc Indian Acad Sci (Anim Sci)*, **93** (1984) 203-23.
5. Nair N B, Vertical distribution of wood-boring animals in Cochin Harbour, *Hydrobiologia*, **27** (1966) 240-59.
6. Nair N B, Seasonal settlement of marine wood boring animals at Cochin Harbour, *Int Revue ges Hydrobiol*, **50** (1965) 34-42.
7. Nair N B, The shipworms of S India with a note on the breeding season of *Bankia indica*, *J Bombay natu Hist Soc*, **54** (1957) 344-57.
8. Nair N B, Ecology of reproduction in tropical shipworms, *Proc 6th Int Cong on Marine Corrosion and Fouling*, Athens, 1984.
9. Nair N B, The development of the wood-boring pelecypod *Bankia indica* (Nair), *J Madras Univ*, **26B** (1956) 303-18.
10. Nair N B, Rate of growth of *Bankia indica* Nair, a shipworm from the Madras coast, *J mar biol Ass India*, **2** (1960) 216-220.
11. Nair N B, The marine timber boring molluscs and crustaceans of W Norway, *Publ Biol Stn Espegrand Univ Bergen Arb Natures Vekke* (1959) 13-23.
12. Nair N B, The problem of timber destroying organisms along the Indian coasts, *Proc 2nd Int Congress Marine Corrosion and Fouling*, Athens, 1968.
13. Nair N B, Sex changes in the wood-boring pelecypod *Bankia indica* Nair, *J Madras Univ*, **26B** (1956) 277-80.
14. Nair N B, The digestive enzymes of *Bankia indica* Nair, *Curr Sci*, **24** (1955) 126-27.
15. Nair N B, The path of enzyme hydrolysis of cellulose in the wood-boring pelecypod, *J scient ind Res*, **15C** (1956) 182-83.
16. Nair N B & Ansell A D, The mechanism of boring in *Zirphaea erispata* (L) (Bivalvia. Pholadidae), *Proc R Soc*, **B170** (1968) 155-73.
17. Nair N B & Ansell A D, Characteristics of penetration of substratum by some marine bivalves, *Proc malac Soc, Lond*, **38** (1968) 1979-87.



## M L Bhatia

Bhatia's main research interest has been in anatomy and functional morphology. His classical work has been on leech, for which he has been referred to as a world authority. His findings have been quoted by zoologists in their monographs, books and research papers published in prestigious journals. The work has covered all aspects of the leech, such as external characters, body walls, alimentary canal, naemocoelomic system, excretory system, nervous system, receptor organs, reproductive systems, bionomics and distribution.

His studies extend to a variety of species, including *Hirudinaria* (Indian cattle leech), *Hirudo medicinalis*, *Haemopsis sanguisuga*, *Hirudinaria granulosa*, *Haemadipsa zeylanica agilis* (Moore), *Haemopsis indicus* Bhatia (Indian carnivorous leech), *Haemopsis indicus* N., *Placobdella emydal* Harding, *Glossiphonia complanata* (Linnaeus), *Theromyzon* sp. nov (Bhatia), *Hemiclepsis marginata* (C R Muller), *Erpobdella octoculata* (Linnaeus), *Glossiphonia lobata* N Sp, etc.

The work on *Hirudinaria*, together with that on *Hirudo medicinalis* and *Haemopsis sanguisuga*, is summarized in his monograph published in 1941<sup>1</sup> and revised in 1984<sup>2</sup>.

The major contributions of Bhatia on leeches are discussed below in brief.

### (1) Segmentation of the Gnathobdellid Leeches

The body of the leech is constituted of a number of anatomical units or somites, each of which is externally manifested as a group of five rings or annuli. The limits of these somites or what may be logically considered as the first or the fifth ring of a somite had long been a subject of controversy. The limits are important, since the anatomical positions of internal organs are described in relation to the somites and since these are used for diagnosing the genera and species. The basis of the segmentation described by Whitman in 1884 was opposed by Moore in 1900, who offered a different system of segmentation. This had caused a great deal of confusion. Bhatia supported Whitman's segmentation and in a paper<sup>3</sup> provided evidence in favour of the earlier segmentation scheme. This was based on embryological studies and several other lines of evidence. Though his description of segmentation is now established on a firm footing, it is somewhat disturbing to the followers of Moor's segmentation scheme, since all the morphological descriptions based on Moor's scheme now need to be revised.

### (2) Haemocoelomic System

It was believed since the end of the last century, that *Hirudo* and the *Hirudinaria*

---

Formerly, Professor of Zoology, University of Delhi, Delhi-110007; Residence : L-23, Hauz Khas Enclave, New Delhi-110016.

do not have true blood vessels and there are only coelomic channels filled with a blood-like red fluid. The exact function of these channels was not clear. However, Bhatia demonstrated the existence of what he termed as the haemocoelomic system, which is analogous to the vascular system. The details of the system comprising longitudinal channels and their branches, together with a capillary system, were elaborated by him<sup>1,2</sup>. He also described the haemocoelomic supply to different organs and the course of circulation of the fluid.

### (3) Nephridial System

Bhatia gave a detailed description of structural and functional organization of the nephridial system of Hirudinaria and Hirudo in 1938<sup>4</sup>. Many controversies regarding the structure and functions of the nephridia were resolved through this work. To mention only one of the important discoveries, the structure and the function of the ciliated organ was ill-understood, and was the subject of much controversy. Some believed the organ to be degenerate and others as actively functional in excretory functions. According to Bhatia's observations, "the ciliated organ instead of serving the excretory function in connection with the nephridium has become subservient to the sinus system, for not only does it manufacture corpuscles, but also serves to keep the fluid in active circulation. This apparent change in function is not surprising when we bear in mind the fact that the sinus system is really a reduced and specialized part of the coelomic cavity; so the funnel here keeps the coelomic fluid moving. The 'funnel', therefore, is far from being degenerate; it has multiplied into numerous small ciliated funnels which

cover a larger area and are much more effective in their ciliary action than a single funnel several times larger." The functions of the nephridial system were further elaborated through later studies<sup>5,6</sup>.

### (4) Prostomial Glands

Ever since the discovery of the prostomial gland and the observations on it by Moquin-Tandon in 1826 and others, it had not been possible for any worker to elucidate the function of these glands. By special experimental techniques, Bhatia was able to determine that the secretion from the prostomial glands makes the plugs which close the cocoon at the anterior and posterior poles of the cocoon<sup>7</sup> and that after a fortnight the plugs become loose and get detached, leaving two outlets in the cocoon through which the young leeches wriggle out.

### (5) Photoreceptors

For the first time it was discovered by Bhatia that the land leeches have segmental papillae serving as extra-ocular photoreceptors, which considerably extend the range of vision<sup>8</sup>. Bhatia has made the interesting suggestion that the quick response to slight changes in the intensity of light by these leeches can be attributed to the existence of super-sensitive phototactile receptors in addition to the normal five pairs of eyes. These permit the leeches to identify the distance and direction of the target and adjust their movement path for interception<sup>9</sup>.

### (6) Reproductive Organs

An important discovery was a protandrous tendency demonstrated by Bhatia in *Placobdella emydae* Harding, a leech parasitic on Indian turtles<sup>10</sup>. The young leeches are essentially males, the



testes being well developed and it is later on with the advancement of age that the animals develop the female organs. This appears to be a contrivance to ensure cross-fertilization.

### (7) Bionomics, Distribution and Morphology of Leeches from Kashmir and Kumaon Hills (UP)

The bionomics and morphology of the leeches from Kashmir and Kumaon Hills were worked out<sup>5,11-14</sup>. Three of the leeches were described for the first time<sup>5,11,12</sup>.

The paper on bionomics and distribution of land leeches of Kumaon hills generated considerable interest among the zoologists, since these are a menace to man and cattle in the area<sup>14</sup>.

### (8) Adaptation and Responses of Land Leeches to External Stimuli

A paper on the adaptation and responses of land leeches to external stimuli in which an attempt was made to correlate the responses to the morphological features had attracted much attention<sup>9</sup>.

In addition to the work on the leeches, Bhatia was the first one to elaborate on the vascular system of the lizard. The description of the arterial system<sup>5,16</sup> and of the venous system<sup>17</sup> had been incorporated in textbooks on the subject.

### Selected Publications

1. Bhatia M K, *Hirudinaria* (The Indian cattle leech), *The Indian Zoological Memoirs*, Vol 8, edited by K N Bahl (Lucknow Publishing House, Lucknow) 1941.
2. *Hirudinaria* (The Indian cattle leech with appendix in *Hirudo medicinalis* and *Haemopsis*

- sanguisuga* (The house leech), *Memoirs of Indian Animal Types*, edited by M L Bhatia, Vol I (Hindustan Publishing Corp, Delhi) 1984.
3. Bhatia M L, The regeneration of the gnathobdellid leeches with special reference to the Indian leech *Hirudinaria* and medicinal leech *Hirudo*, *J Morph*, **132** (1970) 361.
4. Bhatia M L, On the structure of the nephridia and funnels of the Indian leech *Hirudinaria*, with remarks on these organs in *Hirudo*, *Q J microsc Sci*, **81** (1938) 27.
5. Bhatia M L, On *Haemopsis indicus* N. sp.: A new arhynchobdellid carnivorous leech from Kashmir, *Proc natn Acad Sci*, **10** (1940) 133.
6. Bhatia M L, On the nephridial system of the Indian carnivorous leech *Haemopsis indicus* Bhatia, *Proc natn Inst Sci India*, **9** (1945) 7.
7. Bhatia M L, The prostomial glands of the Indian leech, *Hirudinaria granulosa*, *J Morph*, **64** (1939) 37.
8. Bhatia M L, Extra ocular photoreceptors in the land leech, *Haemadipsa zeylanica agilis* (Moore) from Nainital, Almora (India), *Nature, Lond*, **176** (1956) 420.
9. Bhatia M L, Land leeches, their adaptation and response to external stimuli, *Zool polon*, **25** (1975) 31.
10. Bhatia M L, On the anatomical details of *Placobdella emydae* Harding: A leech parasitic on Indian turtles, *Zool Anz*, **91** (1930) 15.
11. Bhatia M L, Sur une nouvelle Hirudinic Rhynchobdelle, *Glossiphonia cruciata*, n sp, *Ann Parasit*, **8** (1930) 121.
12. Bhatia M L, Nouvelle Sengsue *Rhynchobdellide glossiphonia lobata* N sp De L'establissement de pisciculture D'Achha Bal (Kashmir), *Ann Parasit*, **12** (1934) 121.
13. Bhatia M L, Fauna of the Dal Lake, Kashmir, *Bull Dep Zool Punjab Univ*, **2** (1939) 1.
14. Bhatia M L, Bionomics and distribution of the land leeches of Kumaon hills, UP, *J natu Hist Soc*, **70** (1973) 36.
15. Bhatia M L, On the arterial system of the lizard *Uromastic hardwickii* Gray, *J Morph Physiol*, **48** (1929) 281.
16. Bhatia M L & Dayal J, On the arterial system of the lizard *Hemidactylus flaviviridis* Ruppel (the wall lizard), *Anat Anz*, **76** (1933) 27.
17. Bhatia M L, The venous system of a lizard *Uromastix hardwickii* Gray, *Zool Anz*, **85** (1929) 1.

## C M S Dass

In the eukaryotic system, the nuclear organization ciliate of protozoa is unique. Within the same cytoplasmic milieu there are present two types of nuclei different in organization and function. One of them is large both in size and total amount of DNA present—this represents the somatic nucleus or macronucleus—while the other is small and contains only diploid amount (2c) of DNA—this represents the micronucleus with generative functions. This system had attracted the attention of a number of protozoologists, but it is only in recent years that the molecular basis of the difference in the organization and function has become clear to a limited extent. While during binary fission macronucleus divides by simple segregation of DNA into two or more bodies, micronucleus goes through regular mitosis. During sexual reproduction, however, the old macronucleus breaks up and is resorbed, while the micronucleus goes through meiosis and forms the gametic nuclei—and the new macronucleus arises by the differentiation of the division product of zygote nucleus. During this differentiation it was observed by us that there is a progressive increase in the amount of DNA in the macronuclear anlagen. It has been one of our endeavours to understand this phenomenon of differentiation of the macronuclear anlagen. Our earlier studies

on the peritricous ciliate *Vorticella* clearly demonstrated a causal association between the rate of DNA synthesis in the anlagen and the rate of resorption of the old macronuclear fragments. This indicated a possible recycling of breakdown products in DNA synthesis. A more quantitative relationship was established by us using Feulgen microspectrophotometry by measuring the amounts of DNA in the macronuclear anlagen. But a direct evidence of reutilization of the breakdown products down to the level of nucleosides during the rapid DNA synthesis in macronucleus anlagen came from our autoradiographic studies. This proved for the first time the occurrence of salvage pathway in ciliate protozoa. The study of the development of the anlagen in the hypotrichous ciliate like *Stylonychia* also showed that during macronuclear development, there are two cycles of DNA synthesis with an intervening stage where the DNA level comes down to a minimum. Now it is known that the first phase of DNA synthesis is linked with the formation of polytene type chromosomes and the second phase with amplification of a selected region of the genome in somatic differentiation. But such a phenomenon is not seen in all ciliates; that only one continuous synthesis occurs was shown by our work on *Blepharisma* using



autoradiographic technique. This was further substantiated by ultrastructural studies which further showed that unlike in *Stylonychia*, during development there is no polytene stage. The chromatin structure undergoes continuous change with DNA synthesis. Using different inhibitors of protein, RNA and DNA synthesis, it was shown that macronuclear development is closely linked with protein and RNA synthesis and any impairment blocks macronuclear development.

The above extensive studies on macronuclear behaviour led us to ask the question: What is the metabolic contribution of micronucleus during somatic function? It is known to synthesize small amounts of RNA, but there are many situations where an organism can survive without a micronucleus. One of the ways of studying this is to remove surgically the micronucleus, destroy it by X-ray or  $\gamma$ -radiation or by chemical inactivation. The surgical method has been used in ciliates with only one micronucleus, as in *Paramecium*. Radiation introduces unpredictable response. Now laser beam or micro-UV beam is used. But we have developed a method by which using the anticancer compound Cis-platin II, we can destroy micronuclei in multi-micronucleate forms. And we have shown that micronucleus does have some regulatory functions in somatic systems. Concomitantly, it became clear that there is a differential sensitivity between macronucleus and micronucleus. While micronucleus is extremely sensitive, the macronucleus has a very high buffering capacity. This can be accounted for by the occurrence of multiple copies of functional genes in the macronucleus. Our experiments have indicated the occurrence of active repair mechanism of

damaged DNA in macronucleus and absence of the same in micronucleus. Further, there is a threshold level and the macronuclear function is impaired only when it reaches this point. This led us to look for an explanation for senility and aging in ciliates. Our experiments with aging clones in *Stylonychia* have shown that micronucleus ages and becomes non-viable much earlier than macronucleus. Non-viability of micronucleus can be tested at the time of development of the macronuclear anlagen, which, in turn, is derived from the fusion of gametic nuclei after micronuclear meiotic division. The expression micronuclear genomic damage expresses itself at specific stages in development—polytene stage, DNA poor stage and vegetative stage. One can find a direct correlation between the clonal age and the point at which development breaks down. But we also found that germinal aging and somatic aging are very different. Somatic aging takes place much later and it expresses in the changes seen in generation time, irregularities in the division mechanism and a gradual reduction in the survival of members of the clone. Possibly, the earliest expression of somatic aging is seen when there is non-expression of mating reaction between members of complementary mating types, as we know mating type reaction is controlled by macronuclear genes and expresses in the cortical changes that occur.

In the hypotrichous ciliates during S-phase DNA synthesis is not a random process as in other eukaryotes but uniquely, it is sequential. The region of DNA synthesis is marked by the 'replication band' which in Efulgen preparations appears as an unstained region. In our high voltage electron

microscope studies we could trace individual chromatin fibres traversing this zone. Progression of this zone can be studied autoradiographically by pulse labelling and it can also be shown that the DNA synthesized during S-phase gets randomized in the nucleus at the time of division. There were speculations that there could be an orderly distribution of chromatin fibres in the macronucleus during vegetative stages to account for sequential synthesis and occurrence of replication band. But our autoradiographic observation of labelled individuals extending over five generations showed the absence of any orderly distribution of chromatin fibres at any stage.

In addition to our extensive studies on ciliates mentioned above, I have also been interested in the organization of chromatin during the formation of sperms in animals. This is an unique process when DNA packing ratio is very high and arrangement of DNA fibres during its transition from spermatid to sperm was studied by me in the grasshopper and in the Apodans and how it is linked with the transition from moderately basic proteins like histones to highly basic protamines.

Another aspect of my study has been the structure of the ribosomes and the organization of polyribosomes in rat liver. Ours was one of the first studies to show the relationship between messenger RNA and ribosomes to form the polyribosomes in the liver cells.

The above constitute some of the highlights of my research work during the past three decades and I would like to acknowledge the contribution of my research students and others in many of these studies.

## Selected Publications

1. Dass C M S & Seshachar B R, The macronucleus of *Vorticella conyallaria* Linn during conjugation, *J Morph.* **89** (1951) 187.
2. Dass C M S & Seshachar B R, A photometric study of DNA synthesis in regenerating macronucleus of *Epoistylis articulata* From, *Proc natn Inst Sci, India*, **20** (1954) 656.
3. Dass C M S & Ris, Submicroscopic organization of the nucleus during spermiogenesis in the grasshopper, *J Biophys Biochem Cytol.* **4** (1958) 129.
4. Dass C M S & Vimala Devi R, Action of ribonuclease on the nucleic acid system in *Spirostomum ambiguum*, *Q J microsc Sci*, **103** (1962) 37.
5. Dass C M S & Bayley S T, A structural study of rat liver ribosomes, *J Cell Biol*, **25** (1965) 9.
6. Dass C M S & Sapra G R, Effect of B-mercaptoethanol on binary fission in *Stylonychia notophora* (Stokes), *Exp Cell Res*, **43** (1966) 622.
7. Dass C M S & Sapra G R, Organization and development of the macronuclear anlagen in *Stylonychia notophora* Stokes, *J Cell Sci*, **6** (1970) 351.
8. Dass C M S, Sapra G R & Rajesh Kumar, Food vacuole formation and membrane turnover in *Blepharisma musculus* v. *seslachari* Bhandary *Indian J exp Biol*, **14** (1976) 535-43.
9. Dass C M S, Intracellular food channel system in *Stylonychia mytilus*, *Proc First Int Cell Biol Conf*, Boston, USA, 1976.
10. Dass C M S, Chadha R & Sapra G R, Distribution of macronuclear DNA in *Stylonychia mytilus*, An autographic study, *Indian J exp Biol*, **17** (1979) 539-45.
11. Dass C M S & Jangi B S, Ultrastructural organization of the poison gland of the centipede *Scolopendra morsitans* Linn, *Indian J exp Biol*, **16** (1978) 748.
12. Dass C M S, Sapra G R & Rajesh Kumar, Fine structure and nucleic acid synthesis during macronuclear development in *Blepharisma musculus*, *Arch Protistenk*, **126** (1982) 293-308.
13. Dutta D, Sapra G R & Dass C M S, Action of cis-dichlorodiamineplatinum (II) on *Stylonychia mytilus* (Ciliate : Protozoa) : Part I—Differential sensitivity of micronucleus + macronucleus, *Indian J exp Biol*, **20** (1982) 499-506.
14. Nanda N, Dass C M S & Sharma V P, An ultrastructural study on the sporogony of *Plasmodium vivax* in *Anopheles stephensi*, *Indian J Malariol*, **22** (1985) 1-15.



## J S Datta Munshi

The structure and function of the gills and accessory respiratory organs of air-breathing fishes have become of great interest in recent years because of the increasing tempo of research into fish respiration, as also their interesting adaptations and many convergences to the ancestral vertebrates which made the transition from water to land<sup>1-4</sup>.

### Habitat Conditions

Ecological studies have been conducted on swamps, chauris and ponds to analyse the habitat conditions of air-breathing fishes<sup>5</sup>.

*Anabas testudineus*, *Clarias magur*, *Heteropneustes fossilis*, *Monopterus albus* and the four species of *Channa* live in different trophic zones of the swamps and derelict ponds. *Periophthalmus vulgaris* and *Boleophthalmus boddarti* are true amphibious fish spending most part of their lives on the soft muds of the estuaries. The shallow derelict and marshy waters of the tropical world may be taken as the normal habitat of these air-breathing fishes. But most of the species are found in all types of waters.

Recent studies on the environmental features of North Bihar swamps have indicated periodic exposure of water to extreme drought conditions under high atmospheric temperature (30°C), which

causes rapid evaporation of water, leaving a rich humous loamy soil with little or no water. In general, these swamps have very poor oxygen conditions. The lower layers are often deoxygenated. Dissolved free CO<sub>2</sub> up to 45 ppm has been recorded. The bicarbonate alkalinity varies considerably from 75 to 330 ppm in different swamps, tending to produce variations in pH from 7.5 to 9. In certain weed-infested swamps, the chloride content varies from 4.30 to 6.02 ppm. H<sub>2</sub>S gas (16.5-19.34 mg/litre) has often been encountered in these swamps. The overall adverse nature of the swampy environment became more apparent when the diurnal fluctuations in relation to seasonal variations of physico-chemical factors were studied<sup>5</sup>.

### Morphological and Electronmicroscopical Studies of Respiratory Organs

These studies have confirmed the earlier deduction that the air-breathing organs of *Clarias batrachus* and *Heteropneustes fossilis* represent modified gill structure and the suprabranchial chambers and the air-sacs are the extensions of the branchial chamber. The respiratory membranes in these fishes have evolved by the fusion of the primary gill lamellae and abbreviation and shortening of secondary gill lamellae and the earlier hypothesis of Munshi that the respiratory islets are modified lamellar structure has been confirmed by EM

---

Professor & Head, Department of Zoology, Bhagalpur University, Bhagalpur-812007; Residence : Ram Ratan Lane, Adampur, Bhagalpur-812007.

studies. The cells forming the vascular spaces in the dendritic organs and the respiratory islets are typical pillar cells of the gills<sup>6</sup>.

Although the suprabranchial chambers in the Anabantidae group are extensions of branchial chambers and the labyrinthine organs have developed on the epibranchials, the "Respiratory Islets" of the accessory respiratory organs have evolved in different fashions. The hypothesis that the labyrinthine organs and the respiratory islets of the suprabranchial chambers represent modified gill structure is now no longer tenable, as EM studies of Hughes and Munshi<sup>7</sup> revealed that in *Anabas testudineus*, the pillar cells of the air-breathing organs do not have the same relationship as in gills but are modified epithelial cells. Obviously, we are dealing with analogous rather than homologous structures that are used to serve the same function supporting the contiguous vascular units which make up these respiratory organs. Such a supporting function is clearly of importance and during evolution different structural arrangements have been selected to provide surfaces with minimum diffusion barrier between blood and the respiratory medium.

Studies on the ultrastructure of gills of air-breathing fishes have provided fresh knowledge<sup>8</sup>. The earlier hypothesis of Munshi and Singh<sup>9</sup> that the pillar cells are modified smooth muscle cells has been confirmed. New structural elements have been discovered in the pillar cells of *B. boddaerti*, which may be related to their amphibious habit<sup>2</sup>.

## Structural Evolution of Vascular Papillae

The vascular papillae of *Monopterusuchia*, *Channa striata*, *Channa marulius* and *Channa punctata* are sections of capillaries which connect the arterial system of the branchial region with those of the veins of the jugular system and which are specialized for gas exchange.

In *Anabas testudineus*, the respiratory islets are formed of a single layer of biserially arranged sets of parallel blood capillaries. Each capillary is lined by a single row of endothelial cells having tongue like processes which may act as minute valves controlling the flow of blood. This *Anabas* model seems to represent an early stage in the evolution of air-breathing organs of these group of fishes in which arterio-venous connections form straight endothelial tubes situated just below the epithelium. At a later stage, they became arranged in an undulatory fashion as in *Channa* and spiral fashion in *Monopterusuchia*. The endothelial cells protrude into the lumen of the papillae and may act as valves, as in *Anabas*. The functional significance of this wave/spiral like arrangement of capillaries giving rise to a series of vascular papillae seems to be that a greater volume of blood could be accommodated for a given surface of respiratory islets. Moreover, the system provides an efficient mechanism to bring the erythrocytes into contact with the gas exchange surface. The greater capillary length would lead to a longer contact time for equilibration of the blood and respiratory medium. It also increases the resistance to blood flow.



### Morphometrics of Gills and Accessory Respiratory Organs of Air-breathing Fishes

These morphometric studies<sup>10,11</sup> give important information regarding the role of the gas exchange machinery of the amphibious fishes during their growth. From morphometric analysis it was suggested that the gills of *Heteropneustes fossilis* are more efficient than those of *Anabas testudineus* and *Clarias batrachus* in so far as the higher weight group of fishes are concerned. With unit increase in body weight, the gill area of *Anabas*, *Channa punctata*, *H. fossilis* and *M. aculeatum* increases by power of 0.615, 0.592, 0.746 and 0.733 respectively. In *C. punctata*, the gill area per unit body weight decreases at a faster rate ( $b = -0.4$ ) and this suggested that the suprabranchial chambers take part in aquatic respiration also when the fish is inside water, whereas in *Anabas*, the gills alone take part in aquatic gas exchange. In *Clarias* and *Heteropneustes*, the gills and skin take care of the aquatic respiration. Morphometric studies of the air-breathing organs in relation to body weight reveal that the power function of the air-sac in *Monopterus* (= *Amphipnous*) *cuchia* ( $b = 0.797$ ) is greater than that obtained for the air-sac of *Saccobranchus* ( $b = 0.662$ ) and suprabranchial chamber of *Anabas* ( $b = 0.574$ ) and *Channa punctata* ( $b = 0.696$ ). These findings suggest that the air-sac of *Monopterus* (= *Amphipnous*) is efficient in maintaining the increasing  $O_2$  demand of the fish as it grows in size.

### Morphometric Estimation of the Diffusing Capacity of Bimodal Gas Exchange Surfaces of Air-breathing Fishes

Knowledge of the diffusing capacity of the gills and accessory respiratory organs

of these amphibious fishes is a new dimension in the field of respiratory physiology<sup>1,10,11</sup>. The diffusing capacity of the gill of *Anabas testudineus* decreases at a faster rate with increasing body weight than that of *Clarias* and *Heteropneustes*. These findings explain why *Anabas* of higher weight group dies when not allowed to breathe atmospheric air, whereas the gills and skin of *Clarias* and *Heteropneustes* are efficient enough to take care of the total metabolic demands of the fishes as they grow in size. The estimation of the diffusing capacity ( $Dt = 0.02085$ ) of the accessory respiratory organs of the air-sacs of *Monopterus* (*Amphipnous*)<sup>11</sup> is less well suited for oxygen uptake than that of *Anabas* ( $Dt = 0.2286$ ).

### Oxygen Uptake by Gills and Skin in Relation to Body Size of Amphibious Fishes

The rate of oxygen consumption ( $VO_2$ ) through gills and skin was measured in air-breathing fishes of different body weights under two experimental conditions, viz. (i) when access to air was allowed and (ii) when it was prevented<sup>1</sup>. The characteristics of the regression lines relating the logarithm of oxygen consumption to log body weight were calculated by the method of least squares. These physiological studies have revealed that in *Anabas testudineus*, *Channa punctata* and *Clarias batrachus*, the oxygen consumption through gills and gill + skin (*Clarias*) increases by power of 0.67, 0.79 and 0.869 respectively when these fishes had free access to air. Further, lowering of the exponent values was indicated in *Anabas* ( $b = 0.53$ ), *C. punctata* ( $b = 0.62$ ) and *C. batrachus* ( $b = 0.841$ ) when they were not allowed to breathe atmospheric air. The lowering of

the exponent value was perhaps related to the stress condition of the air-breathing fishes when they were not allowed to breathe atmospheric air. The fall in 'b' value due to stress condition in air-breathing fishes is a new phenomenon discovered in the physiology of these fishes.

### **Histo-chemical Studies of the Respiratory Membranes**

Five kinds of specialized cells, viz. (a) mucous cells, (b) acidophil granular cells, (c) basophil mast cells, (d) large bi- or trinucleate glandular cells, and (e) mitochondria rich chloride cells have been reported in the gills and accessory respiratory organs of many air-breathing fishes<sup>8</sup>. The lining is essentially mucous in nature. No surfactant materials could be detected.

### **Enzyme Histochemistry of the Respiratory Muscles**

Gill ventilation is under the influence of buccal pressure and opercular suction pumps. These pumps are operated by means of a series of respiratory muscles. The enzyme histochemistry of these respiratory muscles has opened up a new field of investigation. This study has revealed that the muscles operating these respiratory pumps are composite in nature. Red, white and intermediate muscle fibres have been distinguished in the respiratory muscles, depending on their intensity of reaction for succinic dehydrogenase. It has been noted that the muscles innervated by the facialis nerve are dominated by red fibres, whereas those innervated by trigeminal are dominated by white muscle fibres. The cytochemical differentiation of the muscle

fibres reflects their metabolic activities during gill ventilation.

### **Comparative Studies of the Integuments of Amphibious Air-breathing Fishes**

Comparative studies of integuments of some amphibious fishes have been undertaken to know their role in osmoregulation and respiration<sup>12</sup>. These studies have revealed several new types of skin glands, many of which secrete sulphated mucopolysaccharide. Sulphur could be located autoradiographically in these glands. Their role in wound healing can be very well understood. These studies have revealed karatinization in the skin of *Bagarius bagarius*, where cap-like epidermal structures are formed for protection. Regular karatinization and ecdysis occur in this fish.

### **Haematological Studies**

A series of haematological studies carried out in *Amphipnous*, *Anabas*, *Channa* and *Heteropneustes* have given an indication of the oxygen carrying capacity of blood in relation to body size of these amphibious fishes.

### **Biology and Ecology of Fishes**

Different aspects of the biology of fishes (reproduction, feeding habits, body composition and pollution) have been studied along with the ecology of ponds and swamps.

Presently, research is being conducted on the micro-circulatory system of gills and accessory respiratory organs of air-breathing fishes using methacrylate resin preparations of corrosion replicas of blood capillaries and studying them under SEM. These studies have led to the discovery of shunt pathways of blood circulation in gills



and accessory respiratory organs<sup>14,15</sup>. The functional significance of shunt vessels has been analysed.

The capillary loading ( $V_c/S_a$ ) gives some indication of the surface area available for gas exchange with a given volume of blood. This index ( $V_c/S_a$ ) for labyrinthine organs and suprabranchial chambers comes to 3.85 ml/m<sup>2</sup> and 3.13 ml/m<sup>2</sup> respectively. From these studies it may be concluded that the air-breathing organs of *Anabas* are more efficient in gaseous exchange than the lungs of a Dipnoi *Lepidosiren* (12.37-18.87 ml/m<sup>2</sup>), but less efficient than those of *Monopterusuchia* (2.72 ml/m<sup>2</sup>) and certain mammals like rat (1.20-1.30 ml/m<sup>2</sup>)<sup>16</sup>.

### Conclusion

The transition from water to air-breathing in fishes required many structural and functional modifications in their external gas exchange system. In all probability, these adaptive changes in the system have evolved by different ways in nature over an extended period of vertebrate evolution.

### Selected Publications

1. Munshi, J S D, The structure and function of the respiratory organs of air-breathing fishes of India, *Presidential Address, Section of Zoology, Entomology and Fisheries*, 67th Session, Indian Science Congress Association, 1980, 32-70.
2. Munshi J S D, Gross and fine structure of the respiratory organs of air-breathing fishes, in *Respiration of amphibious vertebrates*, edited by G M Hughes (Academic Press, London) 1976, Chap 4, 73-104.
3. Munshi J S D, The structure, function and evolution of the accessory respiratory organs of air-breathing fishes of India, in *Fortschritte der Zoologie*, Vol. 30, edited by Dunker/Fleischer, *Vertebrate morphology* (Gustav Fischer Verlag Stuttgart, New York), 1985, 353-66.
4. Dutta Hiran M & Munshi J S D, Functional morphology of air-breathing fishes: A review, *Proc Indian Acad Sci (Anim Sci)*, **94**(4) (1985) 359-75.
5. Rai D N & Munshi J S D, Observations on diurnal changes of some physico-chemical factors of three tropical swamps of Darbhanga (North Bihar), India, *Comp Physiol Ecol*, **4**(2) (1979) 52-56.
6. Munshi J S D, Weibel E R, Gehr P & Hughes G M, Structure of the respiratory air-sac of *Heteropneustes fossilis* (Bloch)—An electron microscopic study, *J Linn Soc Lond*, 1986, submitted.
7. Hughes G M & Munshi J S D, Fine structure of the respiratory organs of the climbing perch, *Anabas testudineus*, *J Zool, Lond*, **170** (1973) 201-25.
8. Hughes G M & Munshi J S D, Fine structure of the gills of some Indian air-breathing fishes, *J Morphol*, **160**(2) (1979) 169-94.
9. Munshi J S D & Singh B N, On the microcirculatory system of the gills of certain freshwater teleostean fishes, *J Zool, Lond*, **154** (1968) 365-76.
10. Hughes G M, Dube S C & Munshi J S D, Surface area of the respiratory organs of the climbing perch, *Anabas testudineus*, *J Zool, Lond*, **170** (1973) 227-43.
11. Munshi J S D, Hughes G M, Gehr P & Weibel E R, Structure and morphometry of the air-breathing organs of a swamp mud eel, *Monopterusuchia* (Ham), *J Zool, Lond* (1986) in press.
12. Mittal A K & Munshi J S D, A comparative study of the structure of the skin of certain air-breathing freshwater teleosts, *J Zool, Lond*, **163** (1971) 515-32.
13. Munshi J S D & Hughes G M, Scanning electron microscopy of the accessory respiratory organs of the snake-headed fish, *Channa striata* (Bloch) (Channidae, Channiformes), *J Zool, Lond* (1986) in press.
14. Munshi J S D, Olson K R, Ojha J & Ghosh T K, Morphology and vascular anatomy of the accessory respiratory organs of the air-breathing climbing perch, *Anabas testudineus* (Bloch), *Am J Anat* (August 1986) in press.
15. Olson K R, Munshi J S D & Ghosh T K, Gill microcirculation of the air-breathing climbing perch, *Anabas testudineus* (Bloch): Relationship with the accessory respiratory organs and systemic circulation, *Am J Anat* (1986) in press.
16. Munshi J S D, Ghosh T K & Ahmad M, Capillary loading of the air-breathing organs of *Anabas testudineus* (Bloch), *J exp Biol* (1986) submitted.

## C J Dominic

My investigations centre around reproductive biology and endocrinology, with particular reference to mammals. The main areas of my interest are mammalian reproductive pheromones, mammalian reproduction and fertility control, and the histophysiology of the hypothalamo-hypophyseal complex.

### **Mammalian Reproductive Pheromones**

I have been pursuing my studies on mammalian reproductive pheromones for nearly 25 years, with particular reference to the role of primer pheromones in oestrous cycle and pregnancy in laboratory rodents. Detailed studies have been made on several aspects of the male-induced pregnancy block (the Bruce effect) and the male induction of oestrus (the Whitten effect) in mice. My investigations have revealed that the primer pheromone involved in the Bruce effect is present in the urine of intact males and its production is androgen-dependent. This pheromone is nonvolatile, acts on the females through contact and the vomeronasal organ is involved in its perception. My studies have also revealed that the neuroendocrine cause of the pregnancy block is the depression of hypophyseal prolactin release in newly inseminated females following exposure to the urinary pheromone, with consequent failure of corpus luteum development leading to implantation failure. It appears

likely that the dopaminergic and serotonergic neurons are involved in the transmission of the pheromonal stimulus causing the Bruce effect. It is established that the pituitary-adrenal axis is not involved in the Bruce effect. My studies suggest that the male-originating pheromone involved in the induction of oestrus (the Whitten effect) is volatile, androgen-dependent and acts on the females through the main olfactory system. The pheromone involved in the Bruce effect is distinct from the pheromone involved in the Whitten effect. Another interesting finding is that a contact pheromone is involved in the induction of oestrous cycle disruption in unisexually grouped female mice. I have also provided some evidence in favour of the operation of pheromonal control of reproduction in the wild mouse and the Indian field mouse.

### **Mammalian Reproduction and Fertility Control**

Detailed investigations have been made on reproductive cycles and associated phenomena in Indian bats. The discovery of prolonged storage of spermatozoa in the female genital tract of certain Indian bats suggests that prolonged hibernation, which characterizes temperate zone bats, is not an essential corollary for sperm storage. Another interesting finding is the



incidence of differential rates of fetal growth in two successive pregnancies in the bat, *Taphozous longimanus*. This is a very rare phenomenon in mammals. I have also investigated in some detail the morphology and physiology of the male accessory sex glands and epididymis in mammals, and certain aspects of reproduction in the musk shrew. In addition, detailed studies were made on the effects of vasectomy and several antifertility drugs on the male reproductive organs of the musk shrew, which is a nonscrotal species. These studies have revealed that the musk shrew is insensitive to several drugs with pronounced antispermatogenic effects in scrotal species.

### Hypothalamo-Hypophysial Complex

The response of the avian and mammalian hypothalamic neurosecretory system to several experimental stimuli was studied with a view to gathering information pertaining to the regulation of the antidiuretic hormone. A study of the hypophysial vascularization has revealed the widespread occurrence of two distinct groups of portal vessels in the avian pituitary, which appear to be related to the histological bipartition of the median eminence and of the pars distalis. I have also investigated in some detail the cytology and functional significance of the mammalian pituitary and the physiology of the transplanted pituitary.

### Future Plans

Investigations are in progress on several aspects of the primer pheromonal control of reproduction in mammals, such as pheromone receptors, neural pathways involved in the transmission of pheromonal stimuli, and chemistry, control of

production and assays of primer pheromones. It is also proposed to evaluate the effects of some relatively new antifertility compounds in the musk shrew and to analyse the factors underlying the insensitivity of the musk shrew testis to several antifertility compounds.

### Selected Publications

1. Dominic C J, The origin of the pheromones causing pregnancy block in mice, *J Reprod Fertil*, **10** (1965) 469-72.
2. Dominic C J, Observations on the reproductive pheromones of mice. I. Source, *J Reprod Fertil*, **11** (1966) 407-14.
3. Dominic C J, Observations on the reproductive pheromones of mice. II. Neuroendocrine mechanisms involved in the olfactory block to pregnancy, *J Reprod Fertil*, **11** (1966) 415-21.
4. Dominic C J, Reserpine: Inhibition of olfactory blockage of pregnancy in mice, *Science, NY*, **152** (1966) 1764-65.
5. Dominic C J, Effects of ectopic pituitary grafts on the olfactory block to pregnancy in mice, *Nature, Lond*, **213** (1967) 1242.
6. Dominic C J & Singh R M, Anterior and posterior groups of portal vessels in the avian pituitary, *Gen Comp Endocr*, **13** (1969) 22-26.
7. Dominic C J, Role of pheromones in mammalian fertility, in *Neuroendocrine control of fertility*, edited by T C Anand Kumar (S Karger, Basel) 1976, 236-45.
8. Naik D R & Dominic C J, Functional significance of the cells of the pars anterior of the pituitary gland of the musk shrew (*Suncus murinus* L.), *J Endocr*, **79** (1978) 91-102.
9. Krishna A & Dominic C J, Storage of spermatozoa in the female genital tract of the vesperilionid bat, *Scotophilus heathi*, *J Reprod Fertil*, **54** (1978) 319-21.
10. Singh S K & Dominic C J, Effect of vasectomy on the testis and accessory sex glands of the musk shrew, *Suncus murinus* L., *Endokrinologie*, **77** (1981) 137-46.
11. Singh S K & Dominic C J, Failure of 5-thio-D-glucose to induce antispermatogenic effects in the musk shrew, *Suncus murinus* L., *Biol Reprod*, **24** (1981) 655-59.

12. Krishna A & Dominic C J, Differential rates of fetal growth in two successive pregnancies in the emballonurid bat, *Taphozous longimanus* Hardwicke, *Biol Reprod*, **27** (1982) 351-53.
13. Rajendren G & Dominic C J, Involvement of contact stimuli in the male-induced implantation block (the Bruce effect) in mice, *Anim Reprod Sci*, **7** (1984) 377-83.
14. Rajendren G & Dominic C J, Role of vomeronasal organ in the male-induced implantation failure (the Bruce effect) in mice, *Arch Biol*, **95** (1984) 1-9.
15. Gengrade B K & Dominic C J, Studies on the male-originating pheromones involved in the Whitten effect and the Bruce effect in mice, *Biol Reprod*, **31** (1984) 89-96.



## G P Dutta

### Cytochemistry and Cell Biology of Protozoa

Dutta started his research career with studies on the cytochemistry of protozoa and characterization of cytoplasmic inclusions of parasitic and free-living protozoa belonging to various classes. The results of these studies were presented at the first international congress of protozoology, Prague. The Golgi dictyosomes were identified in all the groups of protozoa and it was emphasized that these dictyosomes represented the true Golgi complex<sup>1</sup>. Earlier investigators had proposed that a variety of argentophilic/osmiophilic inclusions of protozoa, such as walls of the contractile vacuole of ciliates, parabasal bodies of hypermastigote flagellates and stigma of phytoflagellates, were the homologues of the Golgi complex; other workers had reported its absence, particularly in ciliates and opalinids. Based on the original work of Dutta on the cytoplasmic inclusions of protozoa, five comprehensive reviews on cytochemistry and ultrastructure of protozoa were published<sup>2-6</sup>. Some of the findings made using light microscopy have been substantiated by leading electron microscopists.

Specialized cytochemical techniques for application with fluorescence microscopy have been developed: these include the

demonstration of double stranded nature of DNA of plasmodia by acridine orange staining<sup>7</sup>, a fluorescence microscopy method for differentiation of drug resistant strains of plasmodia from sensitive ones<sup>8</sup>, a technique for simultaneous demonstration of nucleic acids and lipids by using a combination of fluorochromes acridine orange and 3,4-benzpyrene<sup>9</sup> and demonstration of neutral polysaccharides after sulphation with acridine orange staining<sup>10</sup>.

### Amoebiasis

A comprehensive multidisciplinary book<sup>11</sup> was published in 1981 based on the work carried out on different aspects of amoebiasis. Extensive studies have been carried out on axenic cultivation of *E. histolytica*, including the improvement of TPS-1 Diamond's axenic medium for *E. histolytica* by incorporating 0.2% L-cysteine HCl and elimination of ascorbic acid that provides stable strongly negative O/R potential in the medium; the replacement of horse serum with bovine serum, which is inexpensive and supports the growth of axenic amoebae. The above improvements in the axenic medium have led to large scale cultivation of *E. histolytica* for the preparation of amoeba antigen for immunodiagnosis of clinical cases<sup>12</sup>.

---

Assistant Director, Division of Microbiology, Central Drug Research Institute, Lucknow-226001; C-17, Vivekanand Puri, Lucknow.

## Malaria

**Chemotherapy and drug resistance:** primate malaria screening models for blood schizontocidal, causal prophylaxis and antirelapse (radical curative) activities have been introduced in the Central Drug Research Institute (CDRI). These experimental malaria models were developed through international collaboration from WHO, WRAIR, NIH, etc. This primate malaria programme has given important leads for the development of new anti-relapse compounds which would be less toxic than primaquine. The use of antibiotics in the control of drug-resistant cases of *P. falciparum* has been proposed and this has emerged as an important line in the field of chemotherapy of malaria<sup>13</sup>.

Experimental studies have shown that rodent malaria parasite *P. berghei* can acquire resistance to most of the drugs available today for the control of malaria.

The studies conducted suggest that mefloquine, which is the most active blood schizontocide for the control of multiple drug resistant *P. falciparum*, can eventually lose its efficacy if it is used in subcurative regimens in man. The best combination against which experimental drug-resistance fails to develop is mefloquine plus Fansider. This combination is recommended for the control of resistant strains of *P. falciparum*.

**Bulk production of malaria antigen for seroepidemiology:** Technology for bulk production of lyophilized antigen suitable for the detection of malaria antibody titres by IHA test in filter paper blood samples collected from malaria endemic areas has been developed. The lyophilized malaria

antigen and joint IHA test protocol developed at CDRI have ensured uniformity of results at various ICMR centres engaged in serology of malaria. Besides IHA test, micro-ELISA for human malaria antibodies had been developed using two simian (*P. knowlesi*, *P. cynomolgi*) and one human malaria antigen (*P. falciparum*). Our studies show that ELISA with all the three antigens is a highly sensitive technique for seroepidemiology of human malaria, the seropositivity with the three antigens being 99-100%<sup>14-16</sup>.

**Host-immunosuppression:** A variety of factors are believed to induce host immunosuppression, e.g. pregnancy, hormones and oral contraceptives. Studies carried out have shown that these factors tend to increase the severity of malaria infection in rhesus monkeys. In a controlled study, pregnancy in *M. mulatta* was found to increase parasite load 2.12-fold in *P. cynomolgi* B and 2.71-fold in *P.c.cynomolgi* infected monkeys. Similarly, female rhesus monkeys administered oral contraceptives in doses just sufficient for contraception for 6 cycles and subsequently infected with *P. cynomolgi* B developed 1.46-fold higher parasitaemia after Norinyl treatment and 1.84-fold higher in Ovral-28 fed monkeys compared to untreated controls. With malaria infection with *P. coatneyi*, the Norinyl treated monkeys developed 1.25-fold higher load of parasitaemia, and Ovral-28 treated animals 1.14-fold higher parasitaemia compared to controls. Likewise, the recrudescence load of parasitaemia in subcured *P. knowlesi* infection in rhesus monkeys was 3.46-5.10-fold higher in progesterone/estrogen treated monkeys. Chronic infections/antimalarial therapy in endemic areas also



tended to produce host-immunosuppression, resulting in low serological IHA titres of malaria antibodies. The mechanisms involved in host-immunosuppression need to be elucidated. However, our studies with oral contraceptive treated monkeys show an impaired blastogenic response of the lymphocytes<sup>17,18</sup>.

*Immune mechanism in malaria:* To elucidate the mechanism of immunity in malaria, models with variable immune status of monkeys have been established by curative or interrupted sub-curative chloroquine therapy of *P. knowlesi* infected monkeys. Radical cure at early infection with chloroquine failed to provide appreciable protection to monkeys upon challenge (1/8 monkeys); passive transfer of their serum also failed to provide protection. In another group, radical cure at high parasitaemia provided partial protection (7/17 monkeys), while radical cure after 25 days of chronicity of infection (induced by subcurative therapy at low parasitaemia) provided still better protection (6/9 monkeys). Dutta<sup>19</sup> had shown that in both these groups, the merozoite blocking antibodies are poorly developed, serum factors fail to transfer immunity and agglutinins, opsonins, lysins and precipitins in the sera of these monkeys were weak or depressed or not demonstrable. The protection afforded to nearly 50% of the monkeys against parasite challenge in these groups thus appears to be mediated largely by active phagocytosis and cell-mediated mechanisms. The clinical immunity evidenced under these conditions, where hyperimmune status does not exist, is mediated by the host's cellular defence mechanisms. The concept of merozoite blocking antibody as a sole factor in malaria immunity thus needed some modification. Recently, the World

Health Organization Scientific Working group (1984) has also agreed with Dutta's<sup>19</sup> hypothesis that non-antibody mediated mechanisms of protection against malaria exist; it has also expressed strong views in favour of the possible role of cell-mediated immunity in protection against malaria. The WHO group also now feels that "effective immunity sometimes correlates better with CMI than with antibody". Though considerable effort and time have been devoted by scientists all over the world during the last decade to the characterization of protective antigens of malaria parasites by immunoprecipitation with monoclonal antibodies against asexual blood stages that principally block invasion or cause agglutination of merozoites *in vitro*, these antigens have not been able to provide effective vaccination, particularly in primate malaria. The antibody mediated immunity based on the characterization of monoclonals by merozoite blocking/agglutination tests have given false leads for successful development of malaria vaccine. Characterization of monoclonals by other antibody tests, e.g. opsonins, lysins, precipitins, might provide useful leads for the identification of protective antigens.

### Selected Publications

1. Dutta G P, The role of cytoplasmic inclusions in protozoa, *Proc 1st Int Conf Protozoology, Prague, 1961, Progress in protozoology* (Publishing House, Czechoslovak Academy of Sciences, Prague) 1963, 259-61.
2. Nath V & Dutta G P, Cytochemistry of protozoa with particular reference to the Golgi apparatus and mitochondria, *Int Rev Cytol*, Vol 13 (Academic Press, New York) 1962, 323-55.
3. Dutta G P, Recent advances in cytochemistry and ultrastructure of cytoplasmic inclusions in *Mastigophora* and *Opalinata* (Protozoa). *Int Rev Cytol*, Vol 36 (Academic Press, New York) 1973, 93-135.

4. Dutta G P, Recent advances in cytochemistry and ultrastructure of cytoplasmic inclusions in Ciliophora (Protozoa), *Int Rev Cytol*, Vol 39 (Academic Press, New York) 1974, 285-343.
5. Dutta G P, Recent advances in cytochemistry and ultrastructure of cytoplasmic inclusions in Sarcodina (Protozoa). I. Anaerobic amoebae, *J scient ind Res*, **36** (1977) 226-40.
6. Dutta G P, Recent advances in cytochemistry and ultrastructure of cytoplasmic inclusions in Sarcodina (Protozoa). Part II—Pathogenic and non-pathogenic free-living amoebae, *J scient ind Res*, **38** (1979) 199-218.
7. Dutta G P, Cytochemical significance of acridine orange staining of human plasmodia with some comments on double stranded DNA, *Histochemie*, **24** (1970) 29-32.
8. Dutta G P, *Plasmodium falciparum*: Nucleic acid and pigment changes in resistant strains produced by antimalarial drugs, *Exp Parasit*, **28** (1970) 412-15.
9. Dutta G P, Simultaneous demonstration of nucleic acids and lipids with fluorescence microscopy, *J Histochem Cytochem*, **19** (1970) 252.
10. Dutta G P, Demonstration of neutral polysaccharides with fluorescence microscopy, using acridine orange, *Nature, Lond*, **205** (1965) 712.
11. Dutta G P, Experimental and clinical studies on amoebiasis (Tata McGraw Publishing Co, Ltd, New Delhi) 1981, pp 391.
12. Dutta G P & Sharma P, Immunodiagnosis of amoebiasis, *J scient ind Res*, **38** (1979) 215-30.
13. Puri S K & Dutta G P, Antibiotics in the chemotherapy of malaria, *Prog Drug Res (Basel)*, **26** (1982) 167-206.
14. Dutta G P, Banyal H S & Kamboj K K, Bonnet monkey (*Macaca radiata*): A host for chronic non-fatal as well as acute *Plasmodium knowlesi* infection, *Indian J med Res*, **76** (1982) 134-40.
15. Dutta G P, Srivastava I K, Sharma Pawan & Agarwal S S, Evaluation of plasmodium cynomolgi B antigen in enzyme linked immunosorbent assay (ELISA) test for human malaria, *Indian J Malariol*, **21** (1984) 71-78.
16. Srivastava I K, Sharma P, Nath A, Agarwal S S & Dutta G P, Enzyme-linked immunosorbent assay test with *P. knowlesi* antigen in diagnosis of malaria, *Indian J med Res*, **77** (1983) 431-36.
17. Kamboj K K & Dutta G P, Severity of blood induced *Plasmodium cynomolgi* B and *P.c. cynomolgi* infection in pregnant rhesus monkeys (*Macaca mulatta*), *Indian J Malariol*, **20** (1983) 1-5.
18. Dutta G P & Kamboj K K, Influence of progesterone and estrogen administration on the recrudescence patterns of *Plasmodium knowlesi* infection in female rhesus monkeys (*M. mulatta*) following initial subcurative chloroquine therapy, *Indian J Malariol*, **21** (1984) 79-88.
19. Dutta G P, Immune mechanism of malaria, *Proc Int Symp Hundred Years of Malaria Research*, 17-19 January 1980, 65-66.



## Asok Ghosh

Despite our general acceptance of the functional uniqueness of the avifauna, before 1970's, our knowledge on avian endocrinology was somewhat fragmentary. In 1977, under the convenorship of Ghosh, the first international symposium on avian endocrinology was held in Calcutta, which brought out synchronization in the disconnected facts of this area, and a new subdiscipline termed as 'avian endocrinology' was established. Subsequently, the second (1980) and the third (1984) international symposia on avian endocrinology were held and currently avian endocrinology occupies an esteemed position in comparative endocrinology.

Though this subsience took full shape during 1977, Ghosh and his collaborators had been working in this subdiscipline since 1957. Ghosh's main research interest rests on the histophysiology of the avian adrenal gland and more specifically on the adrenal medulla. The contributions of Ghosh and his collaborators to the field of avian endocrinology as well as other aspects of vertebrate endocrinology are briefly outlined below.

The findings of Ghosh on avian adrenal medulla have indicated the dynamic behaviour of the catechol hormones. Extreme variation in the relative concentrations of epinephrine in medulla is

a unique feature of birds among other vertebrates. It appears that in avian orders with a primitive heritage, the mechanism of hormonal methylation is not so efficient. An efficacious mechanism is probably established in the evolutionary ascent. This is an outstanding discovery in the realm of 'taxonomic endocrinology' and for this Ghosh got international recognition. Recent exhaustive investigations on the relative concentrations of epinephrine in the adrenal medulla of newly-hatched birds have revealed that the efficacy of the methylating enzyme in newly-hatched birds is just reverse of that in the adults.

His observations on avian epinephrine and norepinephrine have brought out more similarities in action than were suspected. Ghosh's investigations definitely cast doubts on the validity of the usual concept derived from mammalian studies that norepinephrine acts mainly on the cardio-vascular system, whereas epinephrine exerts almost exclusive influence on the carbohydrate metabolism. In birds, norepinephrine appears to be an equally effective 'blood sugar enhancing hormone' compared to epinephrine. A probable mechanism of avian norepinephrine action in hyperglycemic response has also been proposed by him. An almost equal effectiveness of both epinephrine and norepinephrine as pressor hormones has been recorded in the avian group.

The biosynthesis of the catecholamines proceeds along the basic mammalian pattern in the chromaffin cell. Ghosh's investigations revealed that conversion from norepinephrine to epinephrine is controlled by glucocorticoids. The inhibitory role of ascorbic acid in the methylation of adrenomedullary norepinephrine has also been demonstrated.

Observations on the relationship between catecholamines and stressful stimuli (heat and cold stress, population pressure and emotional stress) have been recorded. The heat protective action of norepinephrine has been validated and this information has positive implications for poultry husbandry in tropical countries.

Ghosh's recent investigations have revealed that splanchnic denervation of the adrenal medulla alone does not have any influence on the adrenomedullary catecholamine content. Splanchnic denervation partially prevented reserpine-induced norepinephrine depletion, markedly inhibited resynthesis of adrenomedullary catecholamines after reserpine-induced depletion and almost completely prevented the action of insulin and glucagon, indicating neural regulation of adrenomedullary physiology in birds. These findings have tremendous implications in view of regulating adrenomedullary physiology via the splanchnic nerve.

As a result of Ghosh's exhaustive study on the avian adrenal cortex, a 'cytozonal classificatory system' has been proposed. This cytomorphic zonal concept has had worldwide acceptance. His current investigations on the proliferative activity in the juvenile chick adrenal cortex have revealed that both the cytomorphic zones

(viz. the subcapsular zone and the inner zone) are proliferative.

Ghosh and his group have also actively contributed to the following research areas: Endocrine mechanism of avian migration; Reproductive physiology of tropical birds; Endocrine biology and population control (through chemosterilants) of avian pests; Ecological endocrinology of birds; Hormonal control of the preen gland; Cytophysiology of the avian pineals; Hormonal effect on avian blood pressure homeostasis; Biological control (through hormonal manipulations) of bandicoot rat—an agricultural pest.

Ghosh was invited to contribute chapters to 'International Review of Cytology' and 'Avian Endocrinology'. Some of his findings have a direct applied bearing (in poultry physiology and economic ornithology).

### Selected Publications

1. Mahata S K, Sinhaikim A P & Ghosh A, An autoradiographic study on the proliferative activity in the juvenile chick adrenal cortex, *Basic appl Histochem*, **28** (1984) 375-80.
2. Ghosh A & Banerjee J, Effect of population stress on the histophysiology of avian endocrine organs, *J Yamashina Inst Ornith*, **15** (1983) 156-66.
3. Ghosh A & Banerjee J, Influence of social hierarchy on the adrenal gland of rose-ringed parakeet, in *Endocrinology*, edited by K Shizume, H Imura and N Shimizu (Excerpta Medica, Amsterdam), 1983, 87-95.
4. Kobayashi H, Okawara Y, Maitra S, Sinhaikim A P & Ghosh A, Further studies on Angiotensin. II—Induced drinking in birds, *J Yamashina Inst Ornith*, **14** (1982) 137-42.
5. Chatterjee S & Ghosh A, Influence of experimental scurvy on adrenomedullary catecholamine in mammals and birds, *Cell molec Biol*, **28** (1982) 401-3.



6. Ghosh A & Bhakta D, Behaviour of catechol hormones in the migratory process of *Capella gallinago*, in *Recent advances of avian endocrinology*, edited by C Pethes, P Peczely and F Rudas (Pergamon Press) 1981, 393-99.
7. Ghosh A, Structure and function of avian adrenal medulla, in *Avian endocrinology*, edited by A Epple and M H Stetson (Academic Press, New York) 1980, 301-8.
8. Manna C K & Ghosh A, Comparative cytomorphology of the avian adrenocortical tissue, *Z Mikrosk Anat Forsch*, **93** (1979) 104-12.
9. Ghosh A, Cytophysiology of the avian adrenal medulla, *Int Rev Cytol*, **49** (1977) 253-84.
10. Ghosh A & Maiti B R, Effect of social stimulation on the adrenal medulla of the bandicoot rat, in *Neurosecretion—The final neuroendocrine pathway*, edited by F Knowles and L Vollrath (Springer Verlag, Berlin, New York) 1974, 306.
11. Ghosh A, Histophysiology of the avian adrenal medulla, *Presidential Address, Proc 60th Indian Science Congress*, Part II, 1973, 1-24.
12. Ghosh A & Maiti B R, Histochemical alteration in the adrenomedullary hormonal concentration of the bandicoot rats subjected to fighting, in *Histochemistry and cytochemistry*, edited by T Takeuchi, K Ogawa and S Fujita (Japan Society of Histochemistry, Kyoto) 1972, 585-86.
13. Maiti B R & Ghosh A, Probable role of androgen in the regulation of the uropygial gland, *Gen Comp Endocr*, **19** (1972) 527-36.
14. Banerji T K & Ghosh A, The action of reserpine on the head kidney chromaffin cells of some teleosts—A cytochemical study, *Mikroskopie*, **28** (1972) 1-6.
15. Bhattacharyya S P, A comparative study on the histology and histochemistry of uropygial glands, *Cellule*, **69** (1972) 113-26.
16. Bhattacharyya T K & Ghosh A, Influence of surgical and steroidal bursectomy on the behaviour of adrenal ascorbic acid during stress in juvenile pigeons, *Gen Comp Endocr*, **15** (1970) 420-24.
17. Bhattacharyya T K & Ghosh A, Histomorphologic changes following chronic adrenocortical activation and inhibition in the pigeon, *J Morph*, **130** (1970) 257-70.
18. Ghosh A & Datta B, Effect of reserpine on the release of adrenomedullary catecholamines and their role in glycemic and pressor responses in two avian species, in *Progress in comparative endocrinology*, II, edited by M R N Prasad (Academic Press, New York), 1969, 354-57.
19. Kripalani K, Ghosh A & Rahman H, Hypothalamic neurosecretion in relation to water deprivation in ploveids of arid and swampy zones, *J Morph*, **123** (1967) 35-42.
20. Ghosh A, A comparative study of the histochemistry of the avian adrenals, in *Progress in comparative endocrinology*, I, edited by K Takewaki (Academic Press, New York), 1962, 75-80.
21. Ghosh A, Bern H A, Ghosh I & Nishioka R S, Nature of the inclusions in the lumbosacral neurons of birds, *Anat Rec*, **143** (1962) 195-217.
22. Eidingen D & Ghosh A, The effect of mucolytic enzymes on tissues stained with the periodic acid-Schiff technique, *J Histochem Cytochem*, **4** (1956) 200-7.

## S S Guraya

For the last 28 years, Guraya has been actively engaged in research on the comparative reproductive physiology of fish, amphibians, reptiles, birds (including poultry), mammals (including sheep, goat, buffalo and humans), protochordates and some invertebrates. Deeper understanding of their reproductive physiology is the key to human health (in terms of fertility and sterility problems) and development of animal industry, as it helps us to increase their reproductive efficiency, to control the increasing populations of vertebrate pests, to conserve the endangered animal species and to study the effects of environmental pollutants. In summary, Guraya's research findings in comparative reproductive physiology have contributed greatly to progress in human fertility and sterility and in farm animal production, control of vertebrate pests (harmful birds and rodents) at appropriate timings of the year, elucidation of evolutionary processes, utilization and manipulation of various non-mammalian vertebrate species for commercial purposes, etc. The most significant contributions made by Guraya are related to comparative cellular and molecular aspects of follicular growth and maturation, ovulation, follicular atresia, interstitial gland tissue, corpus luteum formation, regression and function, spermatogenesis and functions of spermatozoa, cellular sites of steroid

hormone synthesis in the gonads of lower chordates, submammalian vertebrates, and mammals, including human and farm animals. The original results of these studies have been published in numerous journals of international repute, prestigious reviews and invited chapters in books published by leading international publishers. Based on his work, Guraya wrote books entitled (1) *Biology of ovarian follicles in mammals* (Springer-Verlag), (2) *Biology of spermatogenesis and spermatozoa in mammals* (Springer-Verlag), and (3) *The cell and molecular biology of fish oogenesis* (S Karger).

### Primordial Oocytes

Primordial (or primary) oocytes of different chordates have been shown to vary greatly in the development, morphology and chemical nature of their ooplasmic structures. The origin, morphology, chemical nature and function of the most controversial ooplasmic organelles, i.e. Balbiani's body or yolk nucleus, have been made clearer than was done by studies of earlier workers.

### Follicular Growth and Maturation

The comparative aspects of developmental processes involved in follicular growth and maturation have been worked out for different groups of



chordates. The comparative origin, structure and chemical nature of zona pellucida and cortical granules about which there has been considerable controversy, have been determined more precisely. These findings are of significance in enabling a better understanding of the fertilization process at cellular and molecular levels. A considerable controversy existed previously about the sites of hormone synthesis in the growing follicles or chordates, including mammals. It has been shown by Guraya that the theca interna cells *in vivo* constitute the site for the biosynthesis of steroid hormones, as they possess the morphological, histochemical and biochemical characteristics of well-established steroid gland cells; the granulosa cells *in vivo* are indeed metabolically active; this activity is clearly indicative of protein rather than steroid hormone synthesis, as shown by their morphological, histochemical and biochemical features. The *in vitro* biochemical studies simply express the biochemical potentials of granulosa cells and not their secretory activity *in vivo*. *In vitro* biochemical studies have shown that androgens are synthesized and secreted by the theca interna of maturing follicle and are aromatized into oestrogens by the granulosa cells.

## Ovulation

Different opinions existed previously about the mechanism of ovulation. The results of various studies have shown that the rupture of a follicle (or ovulation) is the result of a series of definitive degraded changes in the wall of follicle, which are apparently brought about by hydrolases.

## Corpus Luteum Formation (or 'Luteinization') and Regression

Guraya has shown that the appearance of diffusely distributed lipoproteins (or smooth endoplasmic reticulum) demonstrated during the transformation of granulosa cells into luteal cells in several vertebrates, including mammals, can serve as a useful histochemical indicator of this process of luteinization not only in the granulosa lutein cells but also in the other steroid-secreting cells (theca lutein, theca interna, and interstitial gland cells) that develop abundant diffuse lipoproteins. By correlating the results obtained with various ultrastructural, histochemical and biochemical techniques, the sub-cellular basis of steroid hormone synthesis in the steroid gland cells has been made much clearer than it was earlier. The granulosa lutein cells of postovulatory follicles (or corpora lutea) of sub-mammalian vertebrates have also been shown by Guraya to develop the cytological, histochemical and biochemical features of mammalian corpora lutea. But it is still to be determined whether the corresponding cells of sub-mammalian vertebrates produce steroid hormones during the period when the eggs are passing through their oviducts. The corpus luteum regression is accompanied by drastic alterations in organelles, enzymes, lipids, etc.

## Follicular Atresia

The reproductive efficiency of female animal species is closely related to the normal follicular growth and subsequent ovulation. Guraya has demonstrated that follicular atresia is a widespread degenerative phenomenon in the ovaries of fish, amphibians, reptiles, birds and

mammals, including humans and farm animals, by which majority of developing eggs at various stages of their development are lost in the ovaries other than ovulation, thus leading to either low fertility or complete sterility; buffalo ovaries during anoestrus have been shown to have extensive degeneration of egg-follicles and hence wastage of eggs within the ovaries other than ovulation. Guraya has discussed the role of various factors in follicular atresia, as our knowledge in this regard continued to be very meagre. An in-depth study of the physiological mechanisms revealed by Guraya has demonstrated that the most important cause of follicular degeneration is the lack of proper amount of endogenous gonadotrophic hormone stimulation to maintain the growth of follicles or is due to imperfect balance of various hormones. He has recommended that these degenerating eggs can be saved by administration of exogenous hormones at an appropriate stage in the female reproductive cycle, thus increasing the reproductive efficiency of animals. He has also thrown fresh light on the physiological significance of follicular atresia in the building of interstitial gland tissue from the theca interna and the surrounding stroma of atretic follicles and consequently of the steroid hormones, e.g. oestrogens, progestogens and androgens secreted by the ovary. Its presence has been shown by Guraya even in the ovaries of non-pregnant women, rhesus monkey, cow, buffalo and non-mammalian vertebrates in which it was discounted by several previous workers. The interstitial gland cells of thecal origin are present from young to old age and show cycles of abundance and differentiation correlated with the reproductive age and cycle.

Although they greatly vary in their development and distribution, they have been shown to possess the cytological, histochemical and biochemical features of other well-established steroid-secreting cells (testicular Leydig cells and luteal cells). It has been suggested that their steroid secretory product may be of great physiological significance in the initiation of puberty and cyclic ovarian activity in the female vertebrates.

### **Morphology, Biochemistry, Physico-chemistry, Physiology and Preservation of Buffalo Semen**

In spite of the fact that buffalo is of great economic importance not only in Punjab but also in other states of India as well as in various other parts of Asia, very little was known previously about the basic characteristics of its semen. Due to lack of this fundamental knowledge, it has not been possible to develop effective methods for the preservation of its semen and artificial insemination, which are essential for buffalo production. Guraya and his coworkers have made significant original contributions on the morphology, biochemistry and physiology of buffalo semen. From morphological and biochemical studies, it has been shown that the best quality of buffalo semen can be obtained in spring; its quality becomes very poor in summer and rainy season, as the incidence of various abnormalities in the spermatozoa and biochemical changes in the plasma are increased. Various enzymes of glycolysis, Krebs cycle and the pentose phosphate cycle in the buffalo spermatozoa have been worked out in detail. Cold shock has been shown to cause the release of various enzymes, thus affecting their motility and metabolism (or fertilizing ability). A detailed



morphological, biochemical and physiological study of spermatozoa in five modified diluters designated A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub> and CAW has shown that there is significant progressive increase in the percentage of sperm abnormalities and the release of various enzymes from spermatozoa into the media and progressive decrease in the percentage of sperm motility and of the live sperms, with increase in time of storage in the diluters. These results have provided a rational basis for evolving a better semen extender for buffalo. It has also been shown that supplementation of various diluters with the drug chloroquine-diphosphate (a membrane stabilizer) lowers significantly the percentages of sperm abnormalities and the release of various enzymes and increases the percentages of sperm motility and of live sperm in most of the cases. The drug effect varied in different diluters. It was maximum in egg yolk citrate (EYC) diluter and minimum in CAW. The EYC modified by Guraya and his team is now being used for obtaining fertility data before it is recommended for artificial insemination in buffalo.

Guraya and Sidhu have written the book entitled "*Buffalo semen : Morphology, biochemistry, physiology and methodology* (USG Publishers and Distributors, Ludhiana).

### **Comparative Cell and Molecular Biology of Testis and Spermatogenesis in Vertebrates, Including Birds, Sheep, Goat, Buffalo, etc.**

Studies on qualitative and quantitative aspects of spermatogenesis as well as their cytological and histochemical features in vertebrates, including birds, sheep, goat and buffalo have shown that their

reproductive efficiency is greatly affected by degeneration of germ cells at various stages of their development and differentiation. The results of these fundamental studies are of considerable applied value in determining the effects of temperature, light, food, management conditions, etc. on the quality and quantity of semen. Seasonal changes in lipids, carbohydrates, nucleic acids, hormones, enzymes, etc. of testes in the amphibians, fish, reptiles, birds and mammals (sheep, goat and buffalo) have been demonstrated, confirming the effects of environmental factors on their reproductive efficiency. The study of cellular and molecular aspects of spermatogenesis and steroidogenesis in different vertebrate groups has indicated differential metabolism of testicular cells and thereby the physiology of the testis.

### **Development and Maturation of Gonads (Ovaries and Testes) in Mammals**

Guraya has made a thorough study of the developmental and maturational processes of gonads in mammals. These results are of value in assessing the normal or abnormal development of gonads in mammals. It has been shown that the gonads of foetus secrete steroid hormones which bring about the development and maturation of genital tract and its associated glands. The hormones secreted by the maturing gonad initiate puberty or sexual maturation of the animal. Any delay in the synthesis and secretion of steroid hormones can lead to sterility.

### **Sites of Steroid Hormone Synthesis in the Gonads**

Guraya has made an in-depth study of the morphological, biochemical and

physiological characteristics of sites of steroid hormone synthesis in the gonads of various snakes, birds, including poultry, mammals, etc. about which there was a considerable controversy. The results of these studies carried out by Guraya are also of great value in having a better understanding of the effects of various pollutants on the reproductive performance of fish and other animals which are being increasingly exposed to them in both fresh water and sea.

### Effects of Chemosterilants on Reproduction

Alpha-chlorohydrin, a model post-testicular antifertility agent, is a simple organic compound formed by the chemical reaction between glycerol and hydrochloric acid. Alpha-chlorohydrin has been suggested to act as a functional sterilant when administered in low doses, i.e. spermatozoa look normal in histological appearance and motility but do not fertilize ova. The mode of action of alpha-chlorohydrin is very controversial. Morphological, histochemical and biochemical studies carried out by Guraya and his coworkers have indicated that sperm metabolism is not affected directly by alpha-chlorohydrin, but it is the abnormal metabolism of testis and epididymis which is mainly responsible for producing infertile spermatozoa. These results indicate that the drug in small doses can affect cellular metabolism.

### Biochemistry and Metabolism of Helminth Parasites

Guraya has made important contributions to the comparative biology and control of helminth parasites which are known to cause various diseases not only in farm animals but also in humans,

thus causing a great loss to animal industry besides affecting human health. These studies have revealed conspicuous metabolic differences in immature and mature stages of amphistomes; the former are resistant to various chemicals and anthelmintics, which have been shown to cause conspicuous changes in the enzyme activities of helminth parasites. Structural, chemical and physiological differences have been demonstrated in the absorptive and excretory surfaces of different helminths—cestode, trematode, nematode and acanthocephalan. Enzyme activities of phosphatases, which are involved in the transport of substances at the absorptive and excretory surfaces of parasitic helminths, have been shown to be affected by the anthelmintic drugs Bilovon, Mansonil, Vermex, Zaniil, Distodin and carbon tetrachloride. The comparative reproductive biology of helminths has also been investigated in detail with the aim of evolving effective methods for their control by interfering with the reproductive processes. In the acanthocephalans, the complexity of the ovarian structure and processes of oocyte origin, development and maturation have been revealed for the first time. Development of reproductive tissues, gametogenesis and egg formation has been studied extensively both *in vivo* and *in vitro*. Host hormones and other chemicals have been shown to affect the gametogenesis, secretions of accessory sex glands and formation of egg-shell in helminths. Some outstanding contributions in this regard have been summarized in the chapter contributed to Volume III of *Reproductive Biology of Invertebrates* (Oxford & IBH Publishers).

### Selected Publications

1. Guraya S S, Morphology, histochemistry and biochemistry of human ovarian compartments



- and steroid hormone synthesis, *Physiol Rev*, **51** (1971) 785-807.
2. Guraya S S, Morphology, histochemistry and biochemistry of follicular growth and atresia, in *Proceedings of Symposium on Ovogenesis-folliculogenesis*, Nouzilly, *Ann Biol Anim Biochem Biophys (Suppl)*, **13** (1973) 229-40.
  3. Guraya S S, Follicular atresia, in *Proceedings of the Golden Jubilee Symposium on Perspectives in Reproductive Physiology of the Female*, Delhi University, *Proc Indian natn Sci Acad*, **39B** (1973) 311-32.
  4. Guraya S S, Gonadotrophins and functions of granulosa and thecal cells *in vivo* and *in vitro*, in *Gonadotrophins and gonadal function*, edited by N R Moudgal (Academic Press, New York) 1974, 280-336.
  5. Guraya S S, Morphology, histochemistry and biochemistry of human oogenesis and ovulation, in *International Review of Cytology*, Vol 37, edited by G H Bourne and F J Danielli (Academic Press, New York), 1974, 121-51.
  6. Guraya S S, Recent advances in the morphology, histochemistry and biochemistry of steroid-synthesizing cellular sites in non-mammalian vertebrate ovary, in *International Review of Cytology*, Vol 4, edited by G H Bourne and F J Danielli (Academic Press, New York), 1976, 365-409.
  7. Guraya S S, Recent advances in the morphology, histochemistry and biochemistry of steroid synthesizing cellular sites in the testes of non-mammalian vertebrates, in *International Review of Cytology*, Vol 47, edited by G H Bourne and F J Danielli (Academic Press, New York), 1976, 99-136.
  8. Guraya S S, Recent advances in the morphology, histochemistry and biochemistry of the developing mammalian ovary, in *International Review of Cytology*, Vol 51, edited by G H Bourne and F J Danielli (Academic Press, New York), 1977, 49-131.
  9. Guraya S S, Recent advances in the morphology, histochemistry and physiology of interstitial gland cells of mammalian ovary, in *International Review of Cytology*, Vol 55, edited by G H Bourne and F J Danielli (Academic Press, New York), 1978, 171-245.
  10. Guraya S S, Recent advances in the morphology, cytochemistry and function of Balbiani's vitelline body in animal oocytes, in *International Review of Cytology*, Vol 59, edited by G H Bourne and F J Danielli (Academic Press, New York), 1979, 249-321.
  11. Guraya S S, Maturation of the follicular wall: Non-mammalian vertebrates, in *The vertebrate ovary*, edited by R E Jones (Plenum Publishing Corporation, New York), 1978, 261-330.
  12. Guraya S S, Recent advances in the morphology and histochemistry of ovulation, in *Recent Advances in Reproduction and Regulation of Fertility*, edited by G P Talwar (Elsevier/North-Holland Biomedical Press, The Netherlands), 1979, 99-105.
  13. Guraya S S, Recent advances in the morphology, histochemistry, biochemistry and physiology of developing and maturing mammalian testis, in *International Review of Cytology*, Vol 62, edited by G H Bourne and F J Danielli (Academic Press, New York), 1980, 187-300.
  14. Guraya S S, Histochemistry of the ovary, in *Biology of the ovary, Chap 3, International Contribution*, edited by ESE Hafez and P Motta (Martinus Nijhoff, The Hague, Netherlands), 1980, 33-51.
  15. Guraya S S & Motta P, Interstitial cells and related structures, in *Biology of the ovary, Chap 5, International Contribution*, edited by ESE Hafez and P Motta (Martinus Nijhoff, The Hague, Netherlands), 1980, 69-85.
  16. Guraya S S, Spermatogenesis and sperm function in Cephalochordata, in *Reproductive Physiology of Invertebrates*, Vol 11, edited by K G Adiyodi and R G Adiyodi (John Wiley and Sons Ltd, England), 1983, 633-48.
  17. Guraya S S, Oogenesis, oviposition and oosorption in Cephalochordata, in *The Reproductive Biology of Invertebrates*, Vol 1, edited by K G Adiyodi and R C Adiyodi (John Wiley and Sons Ltd, England) 1985, 735-52.
  18. Guraya S S & Parshad V R, Accessory sex glands in platyhelminth, in *Reproductive Biology of Invertebrates*, Vol 3, edited by K G Adiyodi and R G Adiyodi (Oxford & IBH Publishers) 1986, in press.
  19. Guraya S S, Recent progress in the structure, origin, composition and function of cortical granules in animal egg, in *International Review of Cytology*, Vol 78, edited by G H Bourne and F J Danielli (Academic Press, New York), 1982, 257-360.
  20. Guraya S S, Hormonal regulation of follicular growth and differentiation in the mammalian

- ovary, in *Proceedings of the Symposium on Cellular Control Mechanisms* (Bhabha Atomic Research Centre, Trombay, Bombay) 1982, 1-85.
21. Guraya S S, Recent advances in the cellular and molecular biology of ovarian follicles, in *Human fertility, health and nutrition: Impact of molecular and biochemical technology*, edited by J David Puett (United Nations Funds for Population Activities, New York), 1984.
22. Guraya S S, *Biology of ovarian follicles in mammals* (Springer-Verlag, Berlin-Heidelberg-New York-Tokyo), 1985.
23. Guraya S S, *The cell and molecular biology of fish oogenesis* (S Karger) 1986.
24. Guraya S S, *Biology of spermatogenesis and spermatozoa in mammals* (Springer-Verlag) 1987, in press.
25. Sidhu K S & Guraya S S, *Buffalo semen: Morphology, biochemistry, physiology and methodology* (USG Publishers and Distributors, Ludhiana) 1985.



# Ishwar Prakash

The Thar desert is unique from the point of view of its geographic location and its inhabitants being an admixture of Saharian and Malayan elements. Continuing research work carried out since 1952 on various aspects of the desert vertebrates has yielded fruitful results and the Central Arid Zone Research Institute has built up a strong database on these aspects.

## Ecology of Vertebrates

The reconnaissance ecological surveys carried out by me have yielded quantified information on the habitat preferences of various vertebrates, especially reptiles and mammals. The behavioural interactions among various bat species, population dynamics, breeding biology and zoogeography have thrown new light on the understanding of chiropteran biology.

Detailed observation on troupe organization and behavioural interrelationships of two primate species have resulted in a new hypothesis on the fission of social bands of rhesus macaque and langur.

My intensive studies on the desert hedgehogs and shrew have brought to light hitherto unreported information on population structure, behaviour and food and breeding habits of these little known insectivores. Through our extensive and intensive studies on rodent ecology,

behaviour, habitat selection, home ranges, population dynamics and ecological distribution, this mammal group of economic importance is probably the best studied in the Thar desert.

## Olfactory Communication among the Rodents

Out of the 22 species found in the desert region, only three possess a mid-abdominal scent-marking gland. These rodents scent-mark the general area in the territory of their movement. The odour of the sebum exudation of this scent-marking gland serves as a signal for other interspecific as well as intraspecific rodents. Detailed studies on this androgen-controlled behaviour tend to indicate that the main purpose of the scent-marking behaviour is to create confidence in the marking animal in its home range, food reservation, to advertise "ready-to-mate" state of the female and phago-stimulants. Evidence has also been gathered that the scent-marking behaviour is associated with dominance hierarchy and plays an important role in the social organization of these animals in as much as scent-marking has an important role in maintaining intrinsic population control among rodents.

## Wildlife Ecology

With the scientific support provided by our studies on ecological distribution,

habitat preference, social organization and behaviour of Great Indian Bustard, Black Buck, Indian Gazelle, a development plan for augmenting the wildlife resources of the Thar desert has been prepared and a number of sanctuaries and Desert National Parks have been established.

### Rodent Pest Management

Rodents are found in abundance in the desert region and inflict serious losses to the grass resource, standing crops and stored foodgrains. Based on the intensive and extensive work on ecology and behaviour, bait shyness, poison aversion and toxicological aspects, viable and cost effective strategies for the control of rodents in various fields have been standardized and propagated all over the country. As a result of our work on rodents, 'Rodentology' has emerged as a distinct subject.

### Desertification Process

Various processes leading to desertification of the land, their consequences and strategies for combating them have been worked out in detail.

### Selected Publications

1. Prakash I, Breeding of mammals in Rajasthan desert, *J Mamm*, **42**(3) (1960) 386-89.
2. Prakash I, Taxonomical and ecological account of the mammals of Rajasthan desert, *Ann Arid Zone*, **1**(1 & 2) (1962) 142-63; **2**(2) (1964) 150-61.
3. Prakash I, Group organization, sexual behaviour and breeding season of certain Indian monkeys, *Jap J Ecol*, **12**(3) (1962) 83-86.
4. Prakash I, Ecology of gerbils of the Rajasthan desert, India, *Mammalia*, **26** (1962) 311-31.
5. Prakash I, Zoogeography and evolution of the mammalian fauna of Rajasthan desert, *Mammalia*, **27** (1963) 342-51.
6. Prakash I, Cytomorphology of the adrenal gland of the Indian desert gerbil, *J Anat Soc India*, **13**(2) (1964) 76-79.
7. Prakash I, Breeding season and litter size of Indian desert rodents, *Z angew Zool*, **58**(4) (1971) 441-54.
8. Prakash I, Gupta R K, Jain A P, Rana B D & Dutta B K, Ecological evaluation of rodent populations in the desert biome of Rajasthan, *Mammalia*, **35**(3) (1971) 384-423.
9. Prakash I & Jain A P, Bait shyness of two gerbils, *Tatera indica* and *Meriones hurrianae*, *Ann appl Biol*, **69**(2) (1971) 169-72.
10. Prakash I & Mathur R P, Efficacy of chlorophacinone for the control of Indian desert rodents, *Pesticides*, **13**(6) (1978) 44-46.
11. Reichman O J, Prakash I & Roig V, Food selection and consumption, *IBP Arid Land Synth*, **16** (1979) 681-716.
12. Prakash I & Kumari Saroj, Occurrence of ventral marking gland in desert rodents, *Saught Mitteil*, **27** (1979) 315-16.
13. Prakash I, Wildlife conservation in the Thar, *Arid Lands Newsleth*, **14** (1981) 2-8.
14. Prakash I & Idris M, Scent marking by the female Indian gerbil, *Tatera indica* from two distinct habitats during oestrus, *Indian J exp Biol*, **20** (1982) 915-16.
15. Prakash I, Asian predators of livestock, Chapter 23, In *Pests, predators and parasites*, edited by W E Howard and R E Marsh (Elsevier Scientific Publishing Company, Amsterdam) 1984, 405-10.
16. Fitzwater W D & Prakash I, *Handbook of vertebrate pest control* (Indian Council of Agricultural Research, New Delhi) 1973, 1-92 (Revised by Prakash I, 1978).
17. Barnett S A & Prakash I, *Rodents of economic importance in India* (Arnold-Heinemann, New Delhi, London) 1975, pp. 175.
18. Gupta R K & Prakash I, *Environmental analysis of the Thar Desert* (English Book Depot, Dehradun) 1975, pp. 484.
19. Prakash I & Ghosh P K, *Rodents in desert environments*, edited by I Prakash and P K Ghosh (Dr Junk Verlag b.v., The Hague) 1975, pp. 628.



## V G Jhingran

Jhingran's research interests were confined mainly to three areas of fishery science: (1) Fishery biology; (2) Capture fishery resource management— (a) brackishwater lakes, (b) freshwater reservoirs, (c) riverine fisheries; and (3) Freshwater aquaculture.

### (1) Fishery Biology

Accurate knowledge of age and growth of fish is a *sine qua non* for management of commercial fisheries based on the species concerned, both capture and culture. Various age indicators, notably markings on fish scales, otoliths, opercular bones, vertebrae centra, etc. are known to be reliable indicators of age in the temperate regions. In the tropics, however, these conventional structures of fish anatomy and morphology have not been generally known to furnish reliable indications of age and growth.

Jhingran's work<sup>1,2</sup> on the Indian major carp, *Cirrhinus mirgala*, from the middle reaches of the River Ganga, has shown that in the tropics too, scales can be reliable indicators of both age and growth. Age and growth of mrigal were deciphered by him using two independent methods of enquiry, viz., scale and length-frequency-distribution studies. It was found that a fairly accurate tally of fish lengths was attained at different ages of the life of the fish, which conclusively proved the issue

under question. Subsequent work, notably on *Catla catla*, by other workers, also proved the point. This study has enabled fishery management of the river stocks of mrigal in which fish ages need to be known in advance.

Length-frequency-distribution studies for age determination of fish suffer from certain drawbacks, mainly in the form of overlaps of fish lengths of different age-groups and year-classes, especially of advanced ones. Jhingran<sup>3</sup> and later Jhingran and Natarajan<sup>4</sup> on the basis of their nine years' fish length data on several commercial species of fish of Lake Chilka, developed and demonstrated a statistical procedure for the analysis and interpretation of length-frequency-distribution data. The procedure comprises initially collecting length data on large samples of randomly picked up fish of different year-classes over several years and superimposing growth curves as lines of best fit. The mid-values of lengths at different ages furnish the average lengths of fish attained at different years of its life regardless of the rate of recruitment and relative success of year-class strengths.

Fool-proof evidence of the correctness of interpretation of length frequency-distribution data as well as scale studies as to derivation of age and growth of fish has been furnished by tagging Chilka fish in

---

Formerly, Director, Central Inland Fisheries Research Institute, Barrackpore; Residence : 132, Indira Nagar Colony, Dehradun-248011.

the lake. Tag and recovery have similarly yielded indisputable evidence of pattern of fish migration in and out of the lake<sup>5,6</sup>. Jhingran's observations<sup>7</sup> in the Chilka lake mouth area furnished further evidence of migration of mature adults of the mullets, notably *Mugil cephalus* and *M. macrolepis* into the sea for breeding.

Jhingran<sup>8</sup> studied the length-weight relationship of the three major carps of India, viz., *Cirrhinus mrigala*, *Labeo rohita* and *Catla catla*. Such studies are helpful in studying fish condition and health and in estimating the total catches of fish in fish markets based on length distribution studies on fish market samples. They also help in constructing analytical mathematical models of fish populations.

Jhingran<sup>9</sup> threw light on the fishery and breeding of the Indian shad *Hilsa ilisha* in freshwaters, a subject of great importance in elucidating certain crucial aspects of the fishery biology of one of the commercially most important migratory fish of India.

## (2) Capture Fishery Resource Management

(a) *Brackishwater lakes* : Jhingran<sup>3</sup> and Jhingran and Natarajan<sup>10</sup>, during their 9 years of research on the fisheries of Chilka Lake in Orissa, studied the problem of fish depletion, a phenomenon of overfishing greatly feared by fishery scientists all over the world. For these investigations, detailed fishery biology of 11 most commercially important species of the lake was studied. The species were the mullets, *Mugil cephalus* and *M. macrolepis*; the threadfin, *Eleutheronema tetradactylum*; the Clupeids, *Hilsa ilisha* and *Nematalosa nasus*; the perches, *Lates calcarifer* and *Gerres setifer*; the Sciaenid, *Pseudosciaena coibor*; the catfish, *Mytus qulio*;

and the shrimps, *Penaeus monodon* and *P. indicus*. The aspects studied were: age, growth, food, maturity, fecundity, migration pattern, mortality rate, recruitment, total catch equated with total fishing effort and catch per unit of effort (CUE). The fluctuations in abundance of these species were also studied in the context of the physico-chemical and hydro-biological parameters of the lake. The relationship of Chilka stocks of fish with those of the same species migrating into the Bay of Bengal in some cases and into the rivers Daya and Bhargavi in others, was also studied. Based on the results of these intensive investigations, it was concluded that the observed fluctuations in catches were due to natural causes which did not amount to depletion and that the maximum sustained yield of fish can be got from the lake if certain elementary conservation measures, such as size and catch limits and fishing-net mesh regulations, are observed and certain developmental measures are adopted. Carefully formulated conservation procedures and developmental measures for sustaining the fisheries of the lake have been duly intimated to the Government of Orissa for implementation.

(b) *Freshwater reservoirs* : Jhingran and Natarajan<sup>11</sup> assessed the fish yielding potential of the DVC reservoirs, Tillaiya and Konar. Physico-chemical and hydrobiological studies were conducted in both and fish catches obtained were correlated in the background of stocking done. The total annual catch and catch per hectare for the years 1968-1971 and the future productivity of both the reservoirs were assessed. These studies have thrown light on the carrying capacities of these freshwater reservoirs.



(c) *Riverine fisheries* : Jhingran<sup>12</sup> elucidated the pattern of the capture fisheries of river Ganga at Buxar, Bihar and on the basis of the results obtained by Jhingran and Chakraborty<sup>13</sup> computed the adverse effects on fish production by the currently followed practice of resorting to highly destructive massive capture fishery of fingerlings of Indian major carps in the river. It has been recommended that as a management practice, the fingerlings captured from the river should be reared further to table size by aquaculture operations rather than marketing them dead as fingerlings. In this manner, colossal losses of fish protein can be avoided and fish production increased considerably.

### (3) Freshwater Aquaculture

Jhingran, Sehgal, Kumar and Ghosh<sup>14</sup> experimented with a freshwater recirculatory biological filter system which has enabled the production of *Labeo rohita* and *Catla catla* fingerlings in a brief span on 32 days against the normal time of 90 days in earth ponds and that too at stocking rates of fry about 250% higher than is normally done in earth ponds.

The system enables economy in water and space; in addition, the biological filter improves the efficiency of production of fingerlings of the Indian major carps, rohu and catla. It is expected that a similar system can be operated for the production of other freshwater and brackishwater species of fish and shrimps. It may also be possible to produce fingerlings 3-4 times in a carp breeding season lasting from June to September in North India and from November to January in Peninsular India. There is also great scope for enhancing the rate of stocking of carp fry in the

recirculatory system to 10 million per hectare.

Polyculture of Indian major carps and selected Chinese carps developed for the first time in India<sup>15</sup>, in the view of Jhingran, is crucial for meeting the problem of shortage of fish protein in India. Extensive freshwater aquaculture can be developed on the basis of regional self-sufficiency in the Indian sub-continent. Intensive polyculture of Indian major carps and Chinese carps has been innovatively termed "Aquaplosion" by Jhingran as a fishery counterpart to green-revolution in agriculture.

Jhingran has contributed 114 scientific papers and has published three books, viz. *Fish and Fisheries of India* (Hindustan Publishing Corporation, Delhi-7, 1975 and enlarged and revised edition 1983: pp. 666); *Coldwater Fisheries of India* (jointly with K L Sehgal) (Inland Fisheries Society of India, Barrackpore 1979: pp. 239); and *A Hatchery Manual for the Common, Chinese and Indian Major Carps* (jointly with R Pullin) (Asian Development Bank, Manila, Philippines and International Centre for Living Aquatic Resource Management, Manila, Philippines, 1985: pp. 191).

In recognition of his contributions on Indian fisheries, especially freshwater aquaculture, Jhingran was given the Rafi Ahmed Kidwai Award (1972-73) and Sunder Lal Hora Memorial Medal (1975-80). In 1977, he was awarded Padma Shri.

### Selected Publications

1. Jhingran V G, Age determination of the Indian major carp *Cirrhina mrigala* (Ham) by means of scales, *Nature, Lond*, **79** (1957) 268-69.

2. Jhingran V G, Studies on the age and growth of *Cirrhina mrigala* (Ham) from the River Ganga, *Proc natn Inst Sci India (B)*, **25**(3) (1959) 107-37.
3. Jhingran V G, *Report on the fisheries of the Chilka Lake* (1957-1960), Central Inland Fish Res Inst, Barrackpore, Bull No 1, (1963), 133.
4. Jhingran V G & Natarajan A V, Derivation of average lengths of different age-groups in fishes, *J Fish Res Bd Canada*, **26**(11) (1969) 3073-76.
5. Jhingran V G & Misha K N, Further fish tagging experiments on the mullet, *Mugil cephalus* Linnaeus, in Chilka lake, *J Bombay natu Hist Soc*, **56**(2) (1959) 226-34.
6. Jhingran V G & Misha K N, Further fish tagging experiments in the Chilka lake, with special reference to *Mugil cephalus* Linnaeus, *Indian J Fish (A)*, (1959).
7. Jhingran V G, Observations on the seaward migration of *Mugil cephalus* Linnaeus from the Chilka lake for breeding, *Curr Sci*, **27**(5) (1959) 181-82.
8. Jhingran V G, General length-weight relationship of three major carps of India, *Proc nat Inst Sci India*, **18**(5) (1952) 449-60.
9. Jhingran V G, Some observations on the hilsa fishery at Buxar (Bihar, India) in the years 1952-54, *Indian J Fish*, **4**(2) (1957) 336-39.
10. Jhingran V G & Natarajan A V, A study of the fisheries and populations of the Chilka lake during the period 1957-65, *J Inland Fish Soc, India*, **1** (1969) 49-126.
11. Jhingran V G & Natarajan A V, An assessment of fisheries of the DVC reservoirs in relation to stocking, *Proceedings of the Seminar on the ecology and fisheries of freshwater reservoirs*, Central Inland Fisheries Research Institute, Barrackpore, 27-29 November 1969, 1978, 429-56.
12. Jhingran V G, The capture fishery of River Ganga at Buxar (Bihar, India) in the year 1952-1954, *Indian J Fish*, **3**(1) (1955) 197.
13. Jhingran V G & Chakraborty R D, Destruction of major carp fingerlings in a section of River Ganga and its probable adverse effects on fish production, *Indian J Fish*, **5**(2) (1958) 291-99.
14. Jhingran V G, Sehgal K L, Kumar K & Ghosh B B, Rearing advanced fry of major Indian carp species in recirculatory-filtering ponds at Barrackpore, West Bengal, *Aquaculture*, **18**(1) (1979) 45-49.
15. Jhingran V G, Systems of polyculture of fishes in the inland waters of India, *J Fish Res Bd Can*, **33**(4) (Pt 2) (1976) 905-10.



## M S Kanungo

The control of infectious and other diseases brought about by advances in medical sciences has caused a continuous increase in the average life span of man; the proportion of old people has been increasing constantly during the last two decades. This is further accentuated by the decreasing birth rate. Hence, increasing number of old people are being supported by decreasing number of younger people. This problem may be alleviated if the basic cause of aging is understood, so that the process of aging that sets in soon after the completion of growth is postponed to a later age, and the duration of youthful, active and productive period is increased. Also, several old age diseases, such as cardiac diseases, arthritis, rheumatism, etc. which set in after 45 or 50 years of age may be postponed to a later age.

Why living organisms age after the cessation of growth is a challenging and basic biological problem. The more or less fixed life span of all individuals of a species, a similar type of decline in various functions after growth, similar life span of identical twins, and children of long lived parents also having long life, point to the fact that aging has a genetic basis. The variations in the life spans of individuals within a species may be due largely to variations in nutrition, stresses of all kinds,

such as temperature, pollution, radiation, etc.

The types of changes that may occur at the level of the gene as an organism passes from the growth phase to senescence were studied indirectly by looking into the changes in various parameters of enzymes, each of which is coded by a specific gene(s). My early work (1964-1976) using rat as the experimental model was concerned with studies on (1) the levels of several enzymes, a large number of which decrease, a few increase and a few others do not change; (2) the levels of certain enzymes which decrease in old age can be raised and brought back to adult levels by administering steroid hormones (estradiol or testosterone); thus their levels are reversible and it is possible to manipulate them; (3) certain enzymes which have two or more subunits occur as isoenzymes (different molecular forms of the same enzyme) having different kinetic properties; they have different types of sub-units that are coded by different genes; our studies showed that different isoenzymes of lactate dehydrogenase and alanine aminotransferase appear in the rat at different phases of the life span, and hence the genes for different subunits are expressed sequentially at different times; (4) immunological, kinetic and peptide mapping of proteins purified from young and old rats showed that the primary

structure or the amino acid sequence of a protein synthesized in old rats is the same as that of the young. From these data it is concluded that if the primary structure of a protein does not change with age, but its level changes, then such a change can be due only to alterations in the expression of the corresponding genes. A model was proposed<sup>8</sup> to explain the mechanism of aging according to which sequential alterations in the regulation of genes brought about by various gene products lead to aging.

In the second phase (1977-85), the predictions made in the model have been tested by looking into the types of changes that occur in the conformation and function of the chromatin, which is a complex of DNA (genes) and histones. The chromatin consists of a string of nucleosomes linked by DNA. Each nucleosome consists of eight molecules of histones around which the DNA is wrapped. It was shown that of the two covalent modifications of histones, acetylation and phosphorylation, decrease with age. Greater acetylation is correlated with greater transcription (expression) of genes. Thus, the decrease in the level of enzymes seen in old age may be related to the inability of histones to undergo acetylation, as it gets increasingly complexed with DNA with age and thereby represses the expression of genes.

That the chromatin becomes more compact with age was shown by the decrease in its digestibility by the endonuclease, DNase I [that cuts DNA at 10 base pair (bp) intervals and its multiples], using isolated nuclei of the brain of young and old rats. The DNA fragments produced were resolved by slab gel electrophoresis. Micrococcal nuclease

that cuts DNA at the internucleosomal region and produces 200 bp (and its multiples) DNA fragments does not show such changes.

Increasing compaction of chromatin should decrease its DNA and RNA synthetic abilities. Using genetic engineering techniques like digestion of chromatin by restriction enzymes, Eco RI, Msp I and Hpa II, followed by nick translation of DNA, it was shown that incorporation of <sup>3</sup>H-dTMP into DNA is greatly reduced in the old. Further, nick translation following digestion by Msp I that cuts both -CCGG- and -CCGG- sequences and Hpa II that cuts only -CCGG- sequence in DNA show that the incorporation after Hpa II digestion is greatly reduced, showing thereby that -CCGG- sequences get increasingly methylated at internal cytosines with age. Since higher methylation of DNA is correlated with decreasing transcription and increasing compaction of chromatin, these studies have established that the DNA in the chromatin gets increasingly complexed with histones due to which the expression of genes decreases. That indeed transcription decreases was shown by measuring the incorporation of <sup>3</sup>H-UMP into messenger RNA using exogenous eukaryotic RNA polymerase II in the presence of  $\alpha$ -amanitin.

Presently, the expression of a few specific genes, thymidine kinase, actin and tubulin, is being studied through Southern and Northern hybridization. Also, since the frequency of cancer is known to increase in old age, the expression of the oncogenes *ras* and *myc*, is also being studied. Once sufficient information on these genes is available, attempts shall be made to manipulate and modulate the



expression of certain key genes using hormones and other effectors in an attempt to slow down or revert the aging process.

Kanungo has supervised the research of 21 PhD candidates. Of his 117 full papers, 98 are on aging. He is the author of the book "Biochemistry of Ageing" (Academic Press, London, 1980), which has also been translated into Russian.

### Selected Publications

1. Kanungo M S, *Biochemistry of ageing* (Academic Press, London) 1980, pp 281.
2. Kanungo M S & Singh S N, Effect of age on the isozymes of lactic dehydrogenase of the heart and brain of rat, *Biochem biophys Res Commun*, **21** (1965) 454-59.
3. Singh S N & Kanungo M S, Alterations in lactate dehydrogenase of the brain, heart, skeletal muscle and liver of rats of various ages, *J biol Chem*, **243** (1968) 4526-29.
4. Kanungo M S & Gandhi B S, Induction of malate dehydrogenase isoenzymes of the livers of young and old rats, *Proc natn Acad Sci, USA*, **69** (1972) 2035-38.
5. Moudgil V K & Kanungo M S, Effect of age of the rat on the induction of acetyl-cholesterase of the brain by  $\beta$ -estradiol, *Biochim biophys Acta*, **329** (1973) 211-20.
6. Kanungo M S, Patnaik S K & Kaul O, Decrease in the level of  $17\beta$ -estradiol receptor in the brain of rats during aging, *Nature, Lond*, **253** (1975) 366-67.
7. Kanungo M S, A model of aging, *J theor Biol*, **53** (1975) 253-61.
8. James T C & Kanungo M S, Effect of sex steroids on CAT and AChE of brain of rats of various ages, *Biochim biophys Acta*, **538** (1978) 205-11.
9. Chainy C B N & Kanungo M S, Induction and properties of pyruvate kinase of the brain of rats of various ages, *J Neurochem*, **30** (1978) 419-27.
10. Thakur M K & Kanungo M S, Modulation of acetylation of chromosomal proteins of the brain of rats of various ages, *Biochem biophys Res Commun*, **81** (1978) 828-31.
11. Kanungo M S & Thakur M K, *Biochem biophys Res Commun*, **87** (1979) 266-71.
12. Kanungo M S, Biochemical and molecular aspects of aging, *Biochem Rev (India)*, **50** (1979) 41-61.
13. Supakar P C & Kanungo M S, Changes in the chromatin of developing rats, *Molec biol Rep*, **9** (1983) 185-90.
14. Chaturvedi M M & Kanungo M S, Analysis of conformation and function of chromatin of brain of young and old rats, *Molec biol Rep*, **10** (1985) 215-19.
15. Chaturvedi M M & Kanungo M S, Analysis of chromatin of the brain of young and old rats by nick translation, *Biochem biophys Res Commun*, **127** (1985) 604-9.
16. Das B R & Kanungo M S, *In vitro* ADP ribosylation of chromosomal proteins of the brain of developing rat, *Molec biol Rep*, in press.

## Krishna Swarup

For the last 15 years, I have been actively engaged in comparative study of the structure and behaviour of the endocrine glands [parathyroid, calcitonin cells, ultimobranchial body (UBB), corpuscles of Stannius (CS) and pituitary prolactin cells] related to serum calcium and inorganic phosphorus regulation in the various vertebrate groups (pisces, amphibia, reptiles, birds and mammals) and have established a school of endocrinology relating to calcium homeostasis at Gorakhpur. The activities of these glands were studied in response to experimental hypercalcemia [induced by PTH, vitamin D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub> and prolactin] and hypocalcemia (induced by calcitonin, glucagon and corpuscles of Stannius-extract) in the various vertebrate groups. (Five species of fishes, three species of anurans, one species each of lizard and snake, four species of birds and seven species of mammals have been worked upon.) Correlative seasonal changes in the activity of these glands in relation to serum calcium and serum inorganic phosphorus levels and the reproductive cycle have also been studied<sup>2-4</sup>. The glandular activities have been established through histochemical studies.

A clear picture of endocrine control of calcium homeostasis has emerged only in the last decade. The main calcium

regulating hormones are: 1,25(OH)<sub>2</sub>D<sub>3</sub>, parathormone and calcitonin.

The physiological role of vitamin D<sub>3</sub> and its metabolites in fishes is poorly understood. Earlier workers, including Urist, MacIntyre and their coworkers, had emphatically denied their role in calcium homeostasis of fishes. We have demonstrated for the first time that vitamin D<sub>3</sub> and its active metabolite [1,25(OH)<sub>2</sub>D<sub>3</sub>] do have a role in calcium homeostasis of fishes<sup>5-7</sup>, as they induce hypercalcemia even in physiological doses.

We also investigated the hypercalcemic effect of vitamin D<sub>3</sub> in *Varanus flavescens* and *Natrix piscator*<sup>8</sup>; no information was available on the effect of vitamin D<sub>3</sub> administration in reptiles.

In fishes, parathyroid is absent and the hypercalcemic factor is released from the prolactin cells. We have made significant contributions in relation to the role of prolactin in the control of calcium metabolism during reproduction in female fish (*Clarius batrachus*) when normal calcium metabolism has been greatly changed. The exogenous administration of this hormone on the histochemical changes of UBB, CS and prolactin cells have also been studied in male *C. batrachus*.



The hypercalcemic effect of prolactin in reptiles has been observed for the first time in *Varanus flavescens*<sup>9</sup>.

Calcitonin is secreted mainly from the thyroid calcitonin (C) cells in mammals. The C-cells originate from neural crest tissue and migrate to UBB. During embryonic development, UBB gets incorporated into the thyroid parenchyma; the C-cells diffuse into the thyroid and the UBB finally degenerates and remains there as ultimobranchial cysts. We have studied the distribution and behaviour of C-cells in a number of mammals with different feeding habits. In house shrew, *Suncus murinus*, we have noticed C-cells in the parathyroid gland<sup>10</sup>. These cells are arranged in two forms—compact and follicular. The follicular arrangement of C-cells is a unique feature among mammals and had not been reported earlier. Further, in *S. murinus*, the ultimobranchial cysts are invariably seen restricted to the parathyroid gland. This is of interest, as in other mammals, these cysts have mainly been reported from the thyroid gland.

We have made a comparative study of the hypocalcemic effect of calcitonin in all vertebrate groups<sup>11-14</sup>.

Glucagon has been reported as a secretagogue for calcitonin leading to hypocalcemia in mammals<sup>15,16</sup>. This effect has, however, not been reported earlier from non-mammalian species. We have reported for the first time the hypocalcemic effect of glucagon in fish, amphibia<sup>17</sup> and birds<sup>18</sup>.

Stannius corpuscles which release hypocalcemic factor(s) in holostean and teleostean fishes are not found in other vertebrates. To satisfy the inquisitiveness whether hypocalcemic factor(s) from fish

CS (an organ absent in non-piscine vertebrates) have the same hypocalcemic effect in other vertebrates, we administered CS extracts to certain anurans, snakes and birds and surprisingly the results were positive and perhaps they are the first reports<sup>19-21</sup>.

Since parathyroid glands make their first phylogenetic appearance in amphibia, it would be worth while to explore the effect of hypophysectomy upon calcium values in adult anurans to ascertain whether pituitary has completely handed over its responsibility of releasing hypercalcemic factor to the parathyroid.

There are no reports regarding work on the following aspects; it would be profitable to undertake studies on them: (i) seasonal fluctuations in reptilian serum calcium levels and the activities of the calcium regulating glands, (ii) effects of PTH and sex hormones on endolymphatic calcium deposits in reptiles, (iii) effects of vitamin D<sub>3</sub> and its metabolites on the bones and scales of fish, (iv) relationship between prolactin and vitamin D<sub>3</sub> metabolism in fishes and lower tetrapods, and (v) role of calcitonin in egg laying birds.

In the early stages, my research interest had been in (i) fishery biology of *Hilsa ilisha* and sexual dimorphism in fishes, and (ii) physiology of reproduction in fishes—correlative cyclic changes in pituitary and gonads of large murels.

### Selected Publications

1. Swarup K & Das V K, Effect of experimental hypercalcemia on the thyroid calcitonin cells of the Indian grey mongoose, *Herpestes edwardsi* (Geoffroy), *Arch. Histol Jap*, **37** (1974) 11-21.
2. Srivastav Ajai K & Swarup K, Seasonal changes in the calcitonin cells and parathyroid gland of

- house shrew, *Suncus murinus*, in relation to serum calcium level, *Zoologischer Anzeiger*, in press.
3. Srivastava S P, Swarup K & Srivastav Shivaji, Seasonal changes in the serum calcium level and prolactin cells of *Clarias batrachus* in relation to the reproductive cycle, *Ann Endocrinol*, in press.
  4. Swarup K, Srivastav S P & Srivastav Ajai K, Seasonal changes in the structure and behaviour of Stannius corpuscles and serum calcium level of *Clarias batrachus* in relation to the reproductive cycle, *Zoologischer Anzeiger*, in press.
  5. Ahmad N & Swarup K, Corpuscles of Stannius of *Mystus vittatus* in relation to calcium and sodium rich environments, *Arch Biol*, **90** (1979) 1-22.
  6. Swarup K & Srivastav S P, Vitamin D<sub>3</sub>-induced hypercalcemia in male catfish, *Clarias batrachus*, *Gen Comp Endocr*, **46** (1982) 271-74.
  7. Swarup K, Norman A W, Srivastav Ajai K & Srivastav S P, Dose dependent vitamin D<sub>3</sub>- and 1,25-dihydroxycholecalciferol-induced hypercalcemia and hyperphosphatemia in male catfish, *Clarias batrachus*, *Comp Biochem Physiol*, **78** (1984) 553-55.
  8. Srivastav Ajai K, Srivastav S P, Srivastav S K & Swarup K, Serum calcium level of the freshwater snake, *Natrix piscator* in response to vitamin D<sub>3</sub> administration, *J Physiol*, **81** (1986) in press.
  9. Swarup K, Srivastav S P, Srivastav Ajai K & Srivastav S K, Calcemic responses of *Varanus flavescens* to prolactin administration, *J Physiol* (1985) in press.
  10. Swarup K, Srivastav Ajai K & Tewari N P, Occurrence of calcitonin cells and cysts in the parathyroid of the house shrew, *Suncus murinus*, *Acta anatomica*, **101** (1978) 340-45.
  11. Swarup K, Tewari N P & Srivastav Ajai K, Effect of salmon calcitonin on serum calcium and inorganic phosphorus levels in parrot, *Psittacula psittacula*, *Arch Biol*, **91** (1980) 15-20.
  12. Swarup K, Tewari N P & Srivastav Ajai K, Response of calcitonin cells, parathyroid glands and bone to prolonged calcitonin administration in the Indian palm squirrel, *Funambulus pennanti* (Wroughton), *Acta anatomica*, **106** (1980) 180-91.
  13. Srivastav Ajai K & Swarup K, Effect of calcitonin on calcitonin cells, parathyroid glands and serum electrolytes in the house shrew, *Suncus murinus*, *Acta anatomica*, **114** (1982) 81-87.
  14. Krishna L & Swarup K, Response of parathyroid glands, ultimobranchial body, paravertebral lime-sacs and serum calcium level to salmon calcitonin administration in *Rana cyanophlyctis*, *Herpetologica* (1985) in press.
  15. Swarup K, Srivastav Ajai K & Tewari N P, Response of calcitonin cells, parathyroid and serum electrolytes during glucagon-induced hypocalcemia in mongoose, *Herpestes edwardsi* (Geoffroy), *Gen Comp. Endocr*, **37** (1979) 541-45.
  16. Swarup K & Srivastav Ajai K, Response of calcitonin cells, parathyroid, serum calcium and serum inorganic phosphorus during glucagon-induced hypocalcemia in the house shrew, *Suncus murinus*, *Cell molec Biol*, **27** (1981) 287-90.
  17. Srivastav Ajai K & Swarup K, Serum calcium and inorganic phosphorus level of *Rana tigrina* in response to glucagon treatment, *Experientia*, **39** (1983) 66-67.
  18. Srivastav Ajai K, Tewari N P & Swarup K, Phosphocalcic response to glucagon treatment in parrot, *Zool Jb Physiol*, **87** (1983) 439-42.
  19. Pandey A K, Krishna L, Srivastav Ajai K & Swarup K, Response of serum calcium to administration of an extract from Stannius corpuscles in the anurans, *Experientia*, **38** (1982) 1314-15.
  20. Srivastav Ajai K & Swarup K, Calcemic response of Stannius corpuscles extract in parrots, *Psittacula psittacula*, *Experientia*, **38** (1982) 869.
  21. Swarup K, Dose-dependent phosphocalcic response of corpuscles of Stannius extract in the freshwater snake, *Natrix piscator*, communicated.



## G K Manna

The research activities of Manna began with his cytogenetical investigations on some orthopteroid insects in 1946 as an MSc student of zoology with thesis in cytology at the University of Calcutta.

### Early Work (1946-1970)

The cytotaxonomy, interrelationships among various groups and mechanisms of chromosomal evolution have been postulated on the basis of the cytogenetical findings of his collaborators and his own and of others in Heteroptera, Homoptera, Gryllidae, Acrididae, Coleoptera, Pisces and Mammalia. These studies also led to important findings, among others, of the structure and behaviour of chromosomes during meiosis in males of different groups of insects and karyomorphology of somatic complements of both sexes of fishes and mammalian species, a number of multiple sex chromosome mechanisms, and peculiar sex chromosome behaviour in some species of Heteroptera, Gryllidae, etc., structural heterozygotes and chromosomal polymorphisms, providing clues to the mechanisms of chromosomal evolution in some species of grasshoppers, gryllids, drosophila, beetles, plant bugs, fishes, mammals, etc. These findings were briefly highlighted in the Presidential Address, Section of Zoology, 56th Indian Science Congress, Bombay<sup>1</sup>.

The contributions on the heteropteran cytogenetics made by Manna<sup>2-4</sup> since 1950 are often quoted in books and research papers and are considered to be classic even now. The invited paper presented by him at the tenth International Congress of Entomology, Canada, 1956<sup>3</sup>, on the cytotaxonomy and interrelationships among various groups of Heteroptera got special mention in *Nature* in its report on the congress for its new approach. Among about 100 species of Heteroptera cytologically investigated by Manna and his collaborators<sup>4</sup>, special mention may be made of the discoveries of unusual occurrence of XO : XX sex mechanism in *Ectrychotes*, and *Polytoxus* in Reduviidae,  $X_1$  and  $X_2$  fusing during anaphase I in *Dysdercus*, sex chromatin elimination in *Iphita*, intra-individual sex chromosomal variations in both sexes of *Lohita*, sex chromosomal variations between Japanese and Indian populations of *Physopelta*, multiple sex chromosome mechanisms in some species of Coreidae, Reduviidae and Nepidae, characteristic chromosome orientation at metaphases I and II, depicting cytotaxonomical parameters along with other features<sup>5,6</sup>, X-ray induced chiasma-like configuration between marker  $X_1$  and Y chromosomes in *Physopelta*<sup>7</sup>, etc.

The most interesting findings from cytogenetical studies on some 70 species

---

Professor, Department of Zoology, University of Kalyani, Kalyani-741235; Residence : P 10/250, Kalyani-741235.

of Coleoptera were the determinate disjunction of X and Y without pairing at metaphase I in *Hyperaspis* and the discovery of 18 out of expected 30 chromosomal types in *Pissodes approximatus* and *P. canadensis* relating to chromosomes A and B, with the possibility of more in *P. terminalis* for polymorphic A, B and C depicting the process of chromosomal evolution in the genus<sup>8</sup>.

In 16 species of short-horned grasshoppers studied, the discovery of the origin of a supernumerary chromosome by degeneration and/or heterochromatinization of a regular member of the genome and the trend of its ultimate elimination in natural populations of *Tristia pulvinata* remained as a unique phenomenon on the origin of a supernumerary chromosome<sup>9</sup>. The hypothesis on the origin of the neo-X and Y chromosomes in sex chromosomally polymorphic species *Euprepocnemis* is a new one.

The study of some 30 species of gryllids revealed structural heterozygote in *Pteronemobius*, *Gryllulus* and *Paranemobius* and the multiple sex chromosomes and their highly peculiar determinate disjunction type behaviour at metaphase I in *Euscyrtus* and *Seychellsia* deserves special mention<sup>10</sup>.

Though over 50 species of homopteran insects showed stereotype meiotic behaviour, the origin of the polymorphic sex chromosomes in *Parabolocratas* remained highly distinct<sup>11</sup>.

Manna reported that chromosomal polymorphisms play a key role in chromosomal evolution not only in natural populations in insects and mammals<sup>1,8</sup> but also in human neoplastic cancer cervix

uteri *in vivo* and in Jensen sarcoma *in vitro*, leading to chromosomal adaptations<sup>12-14</sup>.

Manna undertook human chromosome research in early 1950 and developed a new coumarin technique for handling human chromosomes *in vivo*<sup>15</sup> at a time when it was an enigmatic problem. This technique enabled him to study extensively the chromosomes of several cases of cervix uteri with different grades of cancerous growths<sup>12,15</sup> and to postulate the 'stem line' hypothesis and chromosomal adaptation in human cancer. The handling of human chromosomes *in vivo* still remained difficult, while it has improved marvellously in *in vitro* condition. The cytological contributions of Manna on human non-neoplastic and neoplastic cervix uteri have been referred to in some books. The chromosomal adaptations in cultured cell line of LP<sub>59</sub> and in Jensen sarcoma and its *in vitro* derivatives were also studied by him<sup>13</sup>.

Attention has been paid to experimental cytology since 1962; initially, grasshopper was used as the main experimental model. Later, the X chromosome in the first spermatocyte prophase was used as a novel parameter for assessing various aspects of X-ray-induced chromosome aberrations<sup>1,16</sup> which was appreciated by M J D White (*Animal Cytology and Evolution*) (Cambridge University Press, 1973, 3rd Edn). In later years, using in addition the marker sex chromosomes, X and Y of the X<sub>1</sub>X<sub>2</sub>Y of the male heteropteran *Physopelta* having the so-called 'holocentric' chromosomes, some fundamental similarities with grasshopper having monocentric chromosome were shown as regards non-random distribution of breaks, differential susceptibility of



heterochromatic sex chromosomes and euchromatic autosomes, radioprotection by penicillin, etc. Some of the observations did not bear testimony to the holocentric nature of heteropteran chromosomes<sup>4</sup>.

Study on the effect of odd chemical agents using spermatocyte chromosomes of grasshoppers initiated in 1964 pertained to chromosomal aberrations induced by alterations in pH, treatment with ethyl alcohol, aluminium chloride, colchicine, formalin, 5-bromodeoxyuridine, phenols, maleic hydrazide, insulin, tetracycline, griseofulvin, etc. at a time when not much attention was paid to the assessment of the hazardous effects of chemical agents<sup>1</sup>. These findings led Manna to switch over more vigorously to the study of the environmental pollutants affecting aquatic life using fish as the model and in the terrestrial life using mice as the model.

### Current Work (1971-1985)

After surveying the karyomorphology of more than 50 species of inland fishes starting from 1971<sup>17,18</sup>, Manna and his collaborators chose the exotic fish tilapia (*Oreochromis mossambicus*) as the model<sup>19</sup> and studied the clastogenic and mutagenic potentialities of X-rays, pesticides and some other chemicals using micronucleus test, chromosome aberrations, polyacrylamide gel electrophoresis, progeny testing for lethal effect, etc.<sup>18,19</sup>. Recently, the clastogenic potentiality of living *Pseudomonas aeruginosa* has been shown by the MNT and chromosome aberration studies in Indian carps and tilapia by ip of the log culture of the bacterium. This line of investigation would also be followed in future.

Before switching over to the study of experimental mutagenesis in mammalian model, karyomorphological survey of some 20 species of Indian mammals was conducted<sup>20</sup>; Swiss albino mice were chosen for several reasons<sup>21</sup>. A series of studies on chromosome aberrations induced by the treatment of odd agents revealed mice bone marrow as a very suitable means of testing clastogenic agents<sup>21</sup>. The odd agents tested so far were X-rays among physical agents; the chemical agents tested included (1) nucleic acid components, analogues, etc.—adenine, adenosine, 2-deoxyadenosine, thymidine, uracil, guanine, 5-bromodeoxyuridine, maleic hydrazide and barbitol; (2) phenols—2,4-dinitrophenol, *p*-aminophenol, pyrogallol and gallic acid; (3) substituted amines—hydroxylamine, semicarbazide phenylhydrazine; (4) aziridine group of chemosterilants—apholate, tepa, metapa, thiotepa, ENT 50787, ENT 50172, ENT 51253, ENT 51256, ENT 50990, ENT 50991 and the antimetabolite D-glucoseamine hydrochloride; (5) tuberculostatic drugs—isoniazid, *p*-amino-salicylic acid and thiosemicarbazone; (6) antibiotics—actinomycin D, bacitracin, chloramphenicol, erythromycin, enterocycline, griseofulvin, mitomycin C, neomycin, novobiocin, nystatin, oleandomycin, penicillin, streptomycin, terramycin, tetracycline, and viomycin<sup>22,23</sup>. Among the living mutagens tried were (1) bacteria—*Staphylococcus aureus*, *Streptococcus faecalis*, *Strep. pyogenes*, *Neisseria meningitidis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *S. paratyphi*, *Pseudomonas aeruginosa*, *Sarcina lutea*, *Xanthobacter autotrophicum*, *Spirillum lipoferum* Biezeranke sp., *Bacillus subtilis*; and (2) spores of *Aspergillus fumigatus*, *A. niger*, *A. flavus*.

and *Alternaria brassicicola*. For each species of bacteria, log culture, culture filtrate and isolated bacteria suspended in saline and sometimes heat killed samples were injected intraperitoneally into mice and the bone marrow chromosome aberrations were studied at various intervals; the results have been reviewed from time to time<sup>21,24-26</sup>. The clastogenic potentialities were found for all the bacterial and fungal species, while both the clastogenic and mutagenic potentialities of chemicals—chloramphenicol, griseofulvin and D-glucoseamine hydrochloride and living mutagens—*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Sarcina lutea* and spores of *Aspergillus fumigatus* have been proved by micronucleus test, somatic and germinal chromosome aberrations, mitotic inhibition, sperm head abnormality and sperm depletion test, lethal test at  $F_1$  and  $F_2$  generations, chromosome aberrations in embryos of 15 day gestation and individuals of different ages after parturition at  $F_1$  and sometimes in  $F_2$  generation of the treated line against control lines in mice systems.

The discovery of the mutagenic and clastogenic potentialities of pathogenic and nonpathogenic bacteria and spores of the fungus *Aspergillus* and *Alternaria* made by Manna and his school has been unique in the field of mutagenesis. This discovery has added the new category of mutagens coined by Manna<sup>23-25,30</sup> as 'living mutagens', which has expanded the field of mutagenesis research, the impact of which in the field of environmental mutagenesis hitherto utterly neglected is very great. This would be the field of his future work.

The fundamental discoveries making use of physical, chemical and living mutagens are the similarities in their overall

expressions as individual chromosome aberrations mainly of chromatid type which originate<sup>28</sup> within a few minutes and continue to appear in hour, day, month, etc after treatment of a mutagen and are also seen to continue in  $F_1$  and  $F_2$  generations; breaks are somewhat localized within and between chromosomes which led to the suggestion of the existence of an inherent weaker region<sup>29</sup> akin to fragile sites recently discovered in human chromosomes and the genic control of structural integrity of chromosomes, mutation of which might cause chromosome aberrations of various natures and also lethalties. The inheritance of the mutated gene could be expressed by chromosome aberration/lethals in  $F_1$  and  $F_2$  generations<sup>31</sup>.

### Future Plans

In future, Manna contemplates to continue his research activities broadly in the field of environmutagenesis, in particular on the effects of living mutagens using various microbes on mammalian and fish systems. However, interest on the effects of physical and chemical mutagens would be maintained so as to have an overall view in the field of mutagenesis.

### Selected Publications

1. Manna G K, Some aspects of chromosome cytology, *Presidential Address, Section of Zoology, Proc 56th Ind Sci Cong, Bombay, Pt 2*, 1969, 185-214.
2. Manna G K, A study of chromosomes during meiosis in forty-three species of Indian Heteroptera, *Proc zool Soc, Bengal*, 4(1,2) (1951) 1-116.
3. Manna G K, Cytology and interrelationships between various groups of Heteroptera, *Proc Xth Int Cong Entomol, Canada*, 2 [1958(1956)] 919-34.
4. Manna G K, Chromosomes in evolution in Heteroptera, in *Chromosomes in Evolution in*



- Eukaryote Groups*, Vol 2: edited by A K Sharma & A Sharma (CRC Press, USA), 1984, 189-226.
5. Manna G K, A further evaluation of the cytology and interrelationships between various groups of Heteroptera, *Nucleus*, 5(1) (1962) 7-28.
  6. Manna G K, Evolution of the sex chromosome number and behaviour in Heteroptera, *J Adv Zool*, 3(1) (1982) 20-32.
  7. Manna G K & Dey S K, X-ray induced chiasma-like configurations between the sex chromosomes of the pyrrhocorid bug, *Physopelta schlanbuschi*, *Perspectives in cytology and genetics*, Vol 3, edited by G K Manna and U Sinha (Hindasia Publishers, Delhi) 1981, 367, 71.
  8. Manna G K & Smith S G, Chromosomal polymorphism and interrelationships among bark weevils of the genus *Pissodes* Germar, *Nucleus*, 2(2) (1959) 179-208.
  9. Manna G K & Mazumder S C, Evolution of karyotype in an interesting species of grasshopper, *Tristia pulvinata* Uvarov, *Cytologia*, 32(2) (1967) 236-47.
  10. Manna G K, Chromosome dynamics in Garryloidea, *Nucleus*, 22(2) (1979) 163-74.
  11. Manna G K & Bhattacharjee A K, Meiosis in two species of *Parabolocratrus* with polymorphic sex chromosomes in males of *P. albomaculatus* (Homoptera), *Caryologia*, 26(11) (1973) 1-11.
  12. Manna G K, A study of chromosomes of the human non-neoplastic and neoplastic uterine tissues, *Proc Int Genet Symp, Japan, Cytologia (Suppl)* (1956) 182-87.
  13. Manna G K, Mammalian chromosomes *in vitro*, The chromosomes of Jensen sarcoma and its *in vitro* derivatives, *Nucleus*, 4(1) (1961) 109-24.
  14. Manna G K, Chromosomal polymorphism and cancer, *Proc 3rd All India Cancer Congress*, Calcutta, *Indian J Cancer*, 4(2) (1967) 64-80.
  15. Manna G K, Handling human chromosomes by a coumarin technique, *Stain Technol*, 31(1) (1956) 45-50.
  16. Manna G K, Cytological analysis of the sex chromosomes from the testes cells of grasshopper, *Nucleus*, 10(1) (1967) 64-80.
  17. Manna G K, Cytogenetic studies in fishes and amphibia, in *Genetical research in India, XVth Int Congr Genet*, ICAR, Delhi, 1982, 244-73.
  18. Manna G K, Progress in fish cytogenetics, *Nucleus*, 27(3) (1984) 203-13.
  19. Manna G K, Tilapia fish as a model for testing genotoxic agents, in *Perspectives in cytology and genetics*, Vol 5, edited by G K Manna and U Sinha (All India Cong Cytol, Genet Publ., Kalyani) 1986, 395-406.
  20. Manna G K & Talukder M, Somatic chromosome number in twenty species of mammals from India, *Mamm Chro Newslett*, 17 (1965) 77-78.
  21. Manna G K, Mouse bone marrow as a means of testing clastogenic agents, *Nucleus*, 29 (1986) in press.
  22. Manna G K, Antibiotics in different roles in the induced chromosome aberrations, *Int Seminar on Management of Environment*, BARC, Bombay, 1980, 145-64.
  23. Manna G K, Cytogenetic effects of antibiotics on man and mammals, *Indian Rev Life Sci*, 1 (1981) 189-223.
  24. Manna G K, Bone marrow chromosome aberrations in mice induced by chemical, physical and living mutagens, *Proc 1st All India Cong Cytol, Genet*, Chandigarh, 1971, 144-50.
  25. Manna G K, Chromosome aberrations induced by living mutagens, *Presidential Address, Sect B, 43rd Ann Sess Nat Acad Sci, India*, Jodhpur, 1973, 1-15.
  26. Manna G K, The living mutagens, *Proc natn Acad Sci India, Golden Jubilee Comm Vol*, (1980) 573-606.
  27. Manna G K, The new horizon of mutagenesis, *Curr Sci*, 51(12) (1982) 1087-93.
  28. Manna G K, Induced breakage in chromosomes—Mechanical and/or chemical, *Proc Symp on Structural and Functional Aspects of Chromosomes*, BARC, Bombay, 1975, 194-204.
  29. Manna G K, Nonrandom aberrations and their implication on the probable existence of weaker region in chromosomes, in *Perspectives in cytology and genetics*, Vol 3, edited by G K Manna and U Sinha (All India Cong Cytol, Genet Publ, Kalyani) 1981, 221-33.
  30. Manna G K, Cytogenetic effects in mice induced by living mutagen, *Proc XV International Congress of Genetics, Sec. III E (Part I)* 308.
  31. Manna G K, On the possible involvement of genes in the induction of chromosome aberrations, in *Perspectives in cytology and genetics*, Vol 4, edited by G K Manna and U Sinha (All India Cong Cytol, Genet Publ, Kalyani) 1984, 25-32.

## K N Mehrotra

Major achievements during my professional career of nearly 30 years are discussed below in brief.

### Acetylcholine Metabolism in Insects

The functioning of the nervous system of insects is intimately connected with the metabolism of acetylcholine (ACh). Therefore, its metabolism was studied extensively in various insect species. The existence of ACh in the developing eggs of houseflies and milkweed bug and adult of mite, *Tetranychus telarius*, was established using various pharmacological and biochemical techniques.

Acetylcholinesterase (AChE), the enzyme responsible for the hydrolysis of ACh, was studied exhaustively and was demonstrated to occur in developing eggs of housefly and milkweed bug, larvae of *Chilo zonellus* and *Prodenia litura* and the adults of Indian honey bee and in various parts of the nervous system of desert locusts, besides rats and house sparrow. On the basis of substrate affinity measured using 25 different analogues of acetylcholine, it was shown that there were significant differences at the catalytic sites of the enzyme. The work revealed differences in size and shape of the “esteratic” site, which is the primary target of organophosphate and carbamate types of compounds used in plant protection. Critical differences in the distance between

“esteratic” and “anionic” sites were observed and these could affect the reaction of this enzyme with both substrates and inhibitors. At molecular level, it was shown that AChE occurs in various aggregate forms; the lowest molecular weight or ‘fundamental unit’ had a molecular weight of 80,000 daltons, but the predominant form had a molecular weight of 165,000 or 250,000 daltons, depending upon the insect species. The various multimolecular forms of the enzyme differed in their properties, especially with regard to their reaction to organophosphates and carbamates used in plant protection. This led to the enunciation of what may be termed as the basis for “qualitative selectivity” of the enzyme.

Another line of attack to elucidate the physiological importance of these differences in AChE obtained from various insect and mammalian sources involved the use of various inhibitors, both organophosphates and carbamates. It was established that the AChE from various sources differed in its affinity and reactivity towards inhibitors and aging behaviour. This view was arrived at using 10 different organophosphates and carbamates. For these studies, new methods were evolved and a computer programme was written to provide for clearcut demonstration of these differences. The above work has been quoted extensively in various



textbooks and review articles and has formed the basis of further work in various parts of the world.

The properties of the enzyme responsible for the synthesis of ACh, choline acetyltransferase (ChA) in houseflies, desert locust, mite and rat brain were studied so as to obtain information on the differences which could be utilized for the preparation of "biochemical bullets" selectively effective against insects. By using 17 analogues of choline as substrates, it was shown that the enzymes from different sources have distinctly different substrate affinities. These differences were also revealed in the reaction of the enzyme with sulphhydryl inhibitors. The mechanism of the reaction catalyzed by the enzyme in insects was also shown to be different from that seen in mammals. These differences in the properties of enzymes obtained from mammalian and insect sources have not only been confirmed, but have also led to the synthesis of as many as 200 compounds which could be used as selective inhibitors of this enzyme in insects. Reviewing this work, O'Brien (1967) stated "It is very clear that important differences do exist, and in principle they present an opportunity for designing of selective insecticides".

### Carboxylesterases (EC 3.1.1.1)

Because of the importance of carboxylesterases (CE) in the metabolism of xenobiotics and insecticides, studies were undertaken to demonstrate their presence in *Chilo partellus*, *Dacus dorsalis*, *Schistocerca gregaria*, *Euproctis lunata*, *Lipaphis erysimi*, *Aphis craccivora*, *Brithys crini* and *Heliothis armigera*.

The significant findings from these studies are that the enzyme differs significantly in its substrate specificity from one insect species to another. This difference, perhaps, can explain the differential response of these insects to kinetic properties suggesting that in some insects, for example, *Lipaphis erysimi*, it may not follow normal Michaelis kinetics, whereas normal Michaelis kinetics is seen in the case of *Euproctis lunata*. Even within the same insect species, the enzymes obtained from various organs may differ in their kinetic properties, for example, the CE obtained from the nervous tissues and fat body of *S. gregaria*. The demonstration of CE in the nervous tissues of the desert locust is important and suggests that it may be a built-in safety mechanism for maintaining the integrity of functioning of the nervous system. The CE obtained from *Lipaphis erysimi* was shown to have an extra anionic site. The enzyme occurs in various isomeric forms, which can be demonstrated by polyacrylamide gel electrophoresis in *E. lunata*. Similarly, studies using gel chromatography on Sephadex G-200 revealed that the enzyme may occur in monomeric and trimeric forms in *Chilo partellus*. The predominant esterase had molecular weight of 240,000 daltons, which accounted for nearly 70% activity. The other polymeric forms had molecular weights of 160,000 and 80,000 daltons. These results suggest that the 'fundamental unit' had molecular weight of 80,000 daltons.

### Physiological Effects of Insecticides

Despite the fact that the insecticides are now being used extensively in plant protection work, comparatively little is known about their effects on various non-

target species. Further, meagre information is available on the effects of non-lethal doses of insecticides on the animal population. The results obtained from our studies on the effects of malathion on house sparrow and desert locust suggest that the mechanism of action of this anti-AChE compound differs significantly in these two organisms. The house sparrow is highly susceptible to the insecticide and the toxicity to birds could be correlated to the inhibition of esterases present in the brain. The results with human sparrow also suggest that the nervous system of this animal contains comparatively high amounts of AChE and that nearly 80% of it must be inhibited by the insecticide, at least for 10 min, for it to be toxic. The existence of an endogenous reactivator of bird AChE was inferred from our work. Compared to this, in the case of desert locust, the inhibition of brain AChE was not essential to cause toxicity; instead, the inhibition of AChE in the thoracic ganglion produced the desired physiological effects. An altogether new phenomenon was noted in this regard; it was observed that the desert locust under insecticidal stress releases a number of new proteins into the haemolymph. These proteins were characterized by gel chromatography and gel electrophoresis and were found to have a different molecular weight and Stoke's radius. The results obtained suggest that the insecticides do have an 'inducer effect', which constitutes a real danger from the environment pollution point of view.

### Metabolism of Insecticides

The metabolism of a number of insecticides in the desert locust was studied. Extensive work was done on malathion, dithiolane, diazinon, DDT,

paraoxon and methyl parathion. The metabolism of these insecticides takes place mainly in the fat body of desert locust. It was demonstrated that the enzymes responsible for the activation of organophosphates are different for different insecticides. Malathion activating enzymes are very labile, whereas the enzymes required for the activation of diazinon are very stable. These studies also showed that diazinon activation occurs through the mixed-function oxidase system and that it require NAD or NADP as co-factor for its activity.

DDT is known to be refractory for locusts. It was shown that locusts could tolerate a dose up to 2  $\mu\text{g}$  per g body weight of insect without any detrimental effects. Such a high dose, if given to mammals, would lead to acute poisoning. Detailed studies conducted to elucidate the cause of ineffectiveness of DDT to locust show that it was due to the presence of a very active enzyme, DDT-dehydrochlorinase, in these insects. The half-life of DDT in *Schistocerca gregaria* was shown to the nearly 70 min.

### Host Plant-Insect Pest Interaction

The biochemical basis for host selection by insects was studied extensively and it was shown that the insect-plant relationship is mainly an interaction between the biochemical systems of insects and those of plants, interlinked ecologically. The presence of feeding stimulants for desert locust in the acetone extracts of seven plants was shown. These studies also indicated that the two allied locust species differ in their response to feeding stimulants. A comparison of six edible oils along with wheat bran extracts revealed that various oils differ considerably in their attractiveness to the



two locust species. The oils were fractionated by column chromatography and then various fractions were tested for phagostimulatory activity. It was shown that triglycerides, free sterols, fatty acids and phospholipids were phagostimulatory to locusts. In contrast, the plants *Calotropis gigantea* and *neem* (*Melia indica*) were shown to contain a very active phagodeterrent. The phagodeterrent principle was shown to be mainly due to an alkaloid present in *Calotropis gigantea*, whereas in *neem* it was a triterpenoid, which also had juveno-mimetic effects on *Spodoptera*. These studies also established that there are qualitative and quantitative differences in the food values of plants to the two species of locust. These results, therefore, provided an interlinking of the biochemistry of host plants to that of insects.

### Pheromones and Kairomones

The presence of a powerful sex attractant was demonstrated; it was isolated from the females of the almond moth, *Ephestia cautella*, and was found to attract males of the species. The efficacy of this attractant was successfully demonstrated in a storage godown. These findings have been discussed by Jacobson in his book "Insect Sex Pheromones".

Similarly, the presence of a kairomone for *Bracon brevicornis* in the hexane extract of *Corcyra cephalonica* was demonstrated. The hexane extract of *Corcyra cephalonica* larvae evoked host-seeking response in the parasitoid, *Bracon brevicornis*. The response of parasitoid was given a score of 4 when the kairomone was considered of full strength. Eight day old females of *B. brevicornis* were used for bioassay, because at this

age the maximum number of females elicited the response. The kairomone was a stable preparation and lost nearly 0.5% activity/h under field conditions.

### Selected Publications

1. Mehrotra K N, Development of the cholinergic system in insect eggs, *J Insect Physiol*, **5** (1960) 129-42.
2. Mehrotra K N, Properties of choline acetylase from the house fly, *Musca domestica* L., *J Insect Physiol*, **6** (1961) 215-21.
3. Mehrotra K N & Dauterman W C, The N-alkyl group specificity of choline acetylase from the house fly, *Musca domestica* L. and the two-spotted spider mite, *Tetranychus telarius* L., *J Insect Physiol*, **9** (1963) 293-98.
4. Mehrotra K N & Dauterman W C, The specificity of rat brain acetylcholinesterase for N-alkyl analogues of acetylcholine, *J Neurochem*, **10** (1963) 119-23.
5. Mehrotra K N, Beri Y P, Misra S S & Phokela A, Physiological effects of malathion on the house sparrow, *Indian J exp Biol*, **5** (1967) 219-21.
6. Mehrotra K N, Singh V S & Mookherji P B, Evidence for the presence of a sex attractant in females of almond moth, *Cadra cautella*, *Indian J Ent*, **29** (1967) 304.
7. Mehrotra K N & Phokela A, A comparison of the affinity, phosphorylation and bimolecular rate constants of cholinesterases by organophosphates, *Arch int Physiol Biochem*, **77** (1969) 799-806.
8. Kulkarni A P & Mehrotra K N, Amino acid nitrogen and proteins in haemolymph of adult desert locusts, *J Insect Physiol*, **16** (1970) 2181-99.
9. Singh H N & Mehrotra K N, Esterases during post-embryonic development of the larva of *Chilo zonellus*, *J Insect Physiol*, **16** (1970) 2385-89.
10. Kulkarni A P & Mehrotra K N, Effects of dieldrin and sumiothion on the amino acid nitrogen and proteins in the haemolymph of desert locust, *Schistocerca gregaria* Forsk, *Pesticide Biochem Physiol*, **3** (1973) 420-34.
11. Mane S D & Mehrotra K N, Locust alanine amino transferase has subunit structure, *Experientia*, **32** (1976) 154-55.
12. Dhari K, Prakash N & Mehrotra K N, Localisation and distribution of acetyl-

- cholinesterase (EC 3.1.1.7) in the nervous tissues of desert locust, *Indian J exp Biol*, **14** (1976) 544-49.
13. Mehrotra K N, Selectivity: The aim in insect control by chemicals, *Proc natn Acad Sci*, **46B** (1976) 207-11.
  14. Ajri D S & Mehrotra K N, Localisation and distribution of choline acetyltransferase in nervous tissues of desert locust, *Schistocerca gregaria* Forsk, *Indian J exp Biol*, **15** (1977) 573-75.
  15. Mane S D & Mehrotra K N, Alanine amino transferase: Properties and distribution in the desert locust, *Schistocerca gregaria*, *Insect Biochem*, **7** (1977) 419-26.
  16. Mehrotra K N, Recent advances in biochemistry of the nervous system, of *Schistocerca gregaria* Forsk, in *Recent advances in entomology*, edited by T N Ananthakrishnan (Entomology Research Institute, Loyola College, Madras), 1981, 5-9.
  17. Atma Ram, Tiwari L D, Mehrotra K N & Ram Dass, Evidence for the presence of a kairomone in *Corcyra cephalonica* Stainton larvae for *Bracon brevicornis* Wesmael (Bracenicidner Hymenoptera), *A angew Ent*, **93** (1982) 338-41.
  18. Mehrotra K N & Phokela A, Properties of carboxylesterase (EC 3.1.1.1) from nervous tissues of the desert locust, *Schistocerca gregaria* Forsk, *Proc Indian natn Sci Acad*, **B49** (1983) 393-99.
  19. Mehrotra K N & Phokela A, *Lipaphis erysimi* Kalt carboxylesterase (EC 3.1.1.1) has an anionic site, *Indian J exp Biol*, **23** (1985) 48-50.
  20. Mehrotra K N, Use of DDT and its environmental effects in India, *Proc Indian natn Sci Acad*, **B51** (1985) 169-84.



## Sivatosh Mookerjee

The dominant trend in our laboratory has been to understand the phenomenon of restructuration in different lower eukaryotes. Three model systems have been used to understand the phenomenon at molecular level.

### Subcellular Regeneration in Ciliates

Spirostomum has been found to be an unrivalled unicellular system: when the cell is surgically made into two halves, each half shows a prophetic morphogenetic power of rebuilding the lost subcellular entities within a few hours. There are both macro- and micro-nuclei in each half. By using tritiated thymidine labelling techniques, it has been observed that micronuclei are the seat of intense DNA synthetic activity to rebuild the lost beads of macronuclei. When metabolic inhibitors were used on regenerating spirostomum, inhibitions of RNA and protein synthesis<sup>1,2</sup> like ethidium bromide and chloramphenicol caused profound delay in the regeneration, while potent mitotic poisons like colchicine and mercaptoethanol caused only general injury to the cell. Use of cyanide, sodium azide and iodoacetate further revealed that the energy for regeneration in ciliates like spirostomum is possibly provided by glycolysis rather than aerobic respiration<sup>3</sup>. The role of  $\text{Ca}^{2+}$  and calmodulin is being looked into.

### Sense of Recognition in Sponge Cells

Intricacies of the receptor molecules during reaggregation of the dissociated sponge cells unfold that methyl mannose and glucuronic acid are the key moieties<sup>4,5</sup>. If microtubules of the interacting cells are depolymerized by colchicine and vinblastin, their effects enhance and facilitate aggregation<sup>6</sup>. The synthetic profiles of the dissociated cells show a higher turnover of RNA synthesis. With aggregation, the turnover of RNA is slowed down<sup>7</sup>. Our work shows that not much of new proteins are synthesized from dissociated to aggregated state. Submicroscopic studies have revealed that various types of surface configurations are found between the interacting cells while re-establishing cell contacts<sup>8</sup>.

### Unique Regulative System of Hydra

Hydra continues to be an exemplary model in understanding anteroposterior gradient, regulative phenomenon of cells and molecular intricacies during the rebuilding process of lost structures<sup>9</sup>. Our work has shown that the synthetic profiles of RNA during regeneration of the oral and aboral structures are strikingly dissimilar. It was been established that in all events of regeneration, RNA is called for during the act of resynthesizing the lost structures. By using actinomycin D, masked RNA also

---

Professor, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067; Residence : 2, Dakshinapuram, JNU New Campus, New Mehrauli Road, New Delhi-110067.

has been shown to be present, its half life being more in the aboral region cells than in those belonging to the oral end<sup>10</sup>. Mapping of DNA synthesis along the column of hydra has been made by <sup>3</sup>H-thymidine labelling techniques. The results obtained indicate that regeneration never ends in morphallactic process alone, but is also concerned with active DNA synthesis<sup>11,12</sup>. Excision of oral or aboral structure provides a stimulus to the systems for new synthetic activity of DNA. The profile of synthesis along the column of hydra shows an unlocalized pattern, extending throughout the axis<sup>12</sup>. We have pointed out that always minimum quanta of cells are required to make regeneration possible, below which the fragment cannot grow<sup>13</sup>. By taking into account the specific activity of certain key enzymes, it was found that the somatic replicating system in hydra cannot continue indefinitely. New grounds have been broken in the understanding of the gradient system in quantitative terms. A new model has been proposed to understand the oral-aboral interactions<sup>14,15</sup>. The hypostome is seen to exert stronger control than the basal disc, as shown by using half the midgastric annulus as the perturbing agent. A new area of diphasic difference in hydra in naturally prevailing culture condition taking place almost at the same time of the year has revealed many interesting points. It appears as if there is a genomic switch, which takes place every October till January, when the hydra leads a state of low energy expenditure. The molecular implication of such a depressed phase is that the specific activity of a number of key enzymes like alkaline phosphatase and acid phosphatase is lowered significantly. This has been seen in both whole hydra and in isolates. We are now looking how

again the genome makes the biochemical signal to dedepress and revitalize the different enzymatic networks to regain the usual morphogenetic power of regeneration of the basal disc. Hydrazes collected from different regions of the subcontinent show superficial expression of the morphological non-semblance between the ecotypes. The basic synthetic pattern of DNA/RNA/protein in most of the ecotypes, which are closely related, approximate each other, except for the green hydra, which presents a distinctly different characterization. The results obtained are suggestive that a heat shock type of protein is present in hydra that is controlling the epigenetic expression of the ecotypes under the influence of environmental cues.

### Selected Publications

1. Prusti R, Srinivasan A, Mohanty P & Mookerjee S, Micromolecular constituents of *Spirostomum ambiguum* and their modulations by ethidium bromide and caffeine treatment, *Indian J exp Biol*, **19** (1981) 5-8.
2. Nayak S & Mookerjee S, RNA and protein synthesis during *Spirostomum ambiguum* regeneration, *Indian J exp Biol*, **19** (1981) 203-5.
3. Prusti R K, Reddy A S N & Mookerjee S, Regeneration in *Spirostomum ambiguum*: Effects of some metabolic inhibitors, *Can J Zool*, **62** (1984) 312-14.
4. Kartha S & Mookerjee S, Reaggregation of sponge cells specificity and recovering of adhesion, *Roux Arch Develop Biol*, **185** (1978) 155-65.
5. Kartha S & Mookerjee S, Pattern of tissue reconstruction from sponge aggregations, *Naturwissenschaften*, **65** (1978) 599-600.
6. Kartha S & Mookerjee S, Microtubules depolymerizing drugs enhance cell reaggregation in sponge, *Indian J exp Biol*, **17** (1979) 439-41.
7. Kartha S & Mookerjee S, Regulation of RNA synthesis through cell contact in sponge cells, *Curr Sci*, **48** (1979) 244-46.



8. Kartha S & Mookerjee S, Cell contact in aggregating sponge cells: An ultrastructural study, *Mikroskopie*, **35** (1979) 213-20.
9. Mookerjee S, Macromolecular synthesis in hydroid regeneration, *Proc, Symp Cell Contl Mech*, 1982, 316-20.
10. Venugopal G & Mookerjee S, Macromolecular synthesis and pattern formation in hydras. III. Differential stability of cytoplasmic RNA in regenerating hydras, *Indian J exp Biol*, **18** (1980) 1379-82.
11. Mookerjee S, Activity of the gland cells during the emergence of a new hydra as a bud, *Zool Jb Abat*, **108** (1982) 31-36.
12. Kumar U & Mookerjee S, DNA synthesis during developmental phases of hydra, *Indian J exp Biol*, **21** (1983) 648-52.
13. Nangia P & Mookerjee S, Intervention with transcriptional process during regeneration of midgastric annulus of hydra, *Indian J exp Biol*, **20** (1982) 381-86.
14. Sinha S, Subba Rao J & Mookerjee S, A four-variable model for the pattern forming mechanism in hydra, *Bio Systems (The Salk Inst)*, **17** (1984) 15-22.
15. Sinha S & Mookerjee S, Hydra pattern is controlled by two distinct but interacting morphogen sets, *Roux's Arch Dev Biol*, **194** (1984) 56-60.

## A S Mukherjee

Mukherjee started his research career with Prof. S P Roy-Chaudhuri (1955-59), and worked on recombination in male *Drosophila ananassae*, and on fractional mutations in *Drosophila*. During the next four years, he worked on developmental genetics and genetic basis of differentiation in *Drosophila* under Prof. Curt Stern (1960-64). In 1964-1965, he worked with Prof. W Beermann and demonstrated that the X chromosome of male *Drosophila* makes up its gene dosage difference from that of the female by working twice as hard and synthesizing twice as much RNA. Subsequently, after joining the faculty at the University of Calcutta, he worked on dosage compensation in *Drosophila*, developmental genetics, mechanism of mutation fixation, regulation of gene expression and DNA replication, puffing activity in polytene chromosomes, etc.

Since 1955, Mukherjee has been working uninterruptedly in *Drosophila* genetics and has published pioneering works on at least five fundamentally important genetics and cell biological aspects, namely, genetic control of recombination developmental genetics of *Drosophila*, genetic and molecular basis of dosage compensation and regulation in *Drosophila*, control of DNA replication, and repair system in fixation of mutation, in addition to some significant works in mammalian system, e.g., role of hormones

in development and carcinogenesis. The results of these studies have been published in reputed international journals.

His work on dosage compensation and control of DNA replication has placed Mukherjee's group on international platform. All his works have gained repute and have been confirmed by scientists abroad and cited in nearly 100 reviews and at least half a dozen books (e.g., in the three textbooks by Herskowitz, King and Levine, in *Genetic mosaic and other assays* by C Stern, in *Animal cytology and evolution* by M J D White, in *Cell differentiation* by W Beermann, in *Biology of Drosophila* by Ashburner and Novitski and in *Biology of Drosophila*, edited by Wright and Ashburner). His theory on the male X hyperactivity as the mechanism of dosage compensation has been accepted as Mukherjee's theory of dosage compensation. According to this theory, the single X chromosome of *Drosophila* male compensates the gene products of the diplo X female by hyperactive transcription and a faster rate of DNA growth. Recent evidences obtained by Mukherjee and his group have led to the location of a regulatory modulator type gene on the X chromosome. This modulator gene, perhaps through nonhistone protein, evolves a change in structural organization of the X, specifically in the male, by which it is



rendered into a potentially higher template form. Certain autosomal signals then interact with the template to yield a higher rate of transcription.

Mukherjee's studies on the role of repair system in the fixation of mutations and hormone and cancer research have opened up new vistas in molecular biology and genetics; the latter work has been cited by Dr Robert Holley (Nobel Laureate).

Mukherjee is one of the pioneers in changing the trend of genetical research in animals in India from the conventional classic type to more molecular-biological type and to introduce autoradiography to genetics in India. He discovered the rapid method of scintillation autoradiography. He was invited to attend the second international conference in autoradiography at Edinburgh (1975) as the sole Indian representative. He was a Chairperson in the session on DNA replication at the fourteenth international congress of genetics at Moscow, in 1978. In 1978, Mukherjee's laboratory was identified as one of the selected laboratories in India for UNESCO grant. He has over 200 papers to his credit. Mukherjee has been admitted to the Fellowship of the Indian National Science Academy and the Royal Microscopical Society (Oxon.).

The key features of the achievements of Mukherjee are discussed below.

### (I) Dosage Compensation

Dosage compensation is a phenomenon by which the X coded gene products produced by two dosages of X linked genes in females are equated with that produced by a single dose of the genes in males.

In mammals, this balance is achieved by inactivation of one of the two X chromosomes of the female; thus, the single dose of X linked genes in both sexes remains active and results in equal products (Mary Lyon's hypothesis). In *Drosophila*, I showed that there is no inactivation of X in female, but the balance is achieved by doubling the activity of the single dose of X linked genes in male.

Since this discovery, I have attempted during the last several years to understand the mechanism of regulation of dosage compensation. I with my coworkers have obtained the following results:

#### (1) 1965-1969

(i) The regulation of dosage compensation operates through the male by a positive control device.

(ii) Its operation is seen at the level of transcription.

(iii) Its action is cell autonomous.

(iv) It is not influenced by the developmental rate or the physiology of the sex.

(v) It is independent of sex determining genes.

#### (2) 1970-1972

(i) The hyperactive transcription of the male's single X chromosome parallels an early completion of DNA replication by the X.

(ii) Neither the hyperactive transcription nor the early replication is altered by translocating the position of the X linked genes.

(iii) All these properties could be demonstrated as generally true for many species of the genus *Drosophila*.

(iv) The first indication that the control system of this balance (= dosage compensation) may be through an organizational modulation of the X chromosome was obtained by piecemeal autonomy in polytene chromosome puffs of the X chromosome.

### (3) 1973-1974

(i) Piecemeal autonomy was found to correspond precisely with autonomy in early replication of the X in male.

(ii) The role of the structure of the X in the control of dosage compensation was first proposed in the form of a model.

### (4) 1975-1978

(i) Evidence confirming the validity of the model and some modifications were reported.

(ii) The early completion of the male X was shown to be cell autonomous and it was established that the early replication was due to synchronous initiation followed by faster chain growth of the DNA of the male X chromatin.

(iii) The *modus-operandi* was described in one of the papers.

### (5) 1979-1981

(i) More information on the role of the structural organization of the X chromosome and also of autosomal factors was provided by showing that the X chromosome of the male binds more nonhistone protein than do the female X's, and that secondary signals must come from autosomes.

(ii) The transcriptive activity per gene is increased four-fold in males with duplication for certain small segments of the X chromosome; this finding could be

explained by dual action: (a) modulation of the X chromosomal organization by some regulatory element on X, and (b) interplay of certain autosomal factors. Further proof of this aspect was given by the action of male specific lethals by Lucchesi *et al.*

(ii) Strong support for the joint action came from the *in situ* transcription assay of the X chromosomes using *E. coli* RNA polymerase and *Drosophila* RNA polymerase II. Here, it was shown that even on immobilized chromatin, the male X chromatin utilizes more RNA polymerase than each X chromosome of the female. Thus, the template activity was higher in the male X.

### (6) 1981-1982

(i) Evidence towards the existence of the X linked regulatory gene complex came from the unique superactivity of the X chromosome of a mutant strain *ln(1)BM<sup>2</sup>* (reiv.). In this strain, the X chromosome shows 1.5-2 fold increase in activity over the normal male X. This super-hyperactivity is suppressed when a *B<sup>S</sup>.Y*, i.e., duplication for 16E-17A, is added.

(ii) A model has been proposed for the mode of operation of this complex in the control of dosage compensation.

(iii) Results of the work under way have revealed more important aspects, for example, the extent and operation of the "modulator" factor, the interaction of the factor with autosomal signal, etc.

(iv) It has been observed that the males produce three separate nonhistone chromatin proteins not found in the female. These proteins could be separated by polyacrylamid gel electrophoresis.



## (II) DNA Replication

### (1) 1970-1973

It was established that DNA replication is initiated at multiple initiation points.

### (2) 1974-1976

(i) The sites of replication initiation are organized into early and late replication regions.

(ii) Initiation starts on inter-bands of polytene chromosome of *Drosophila*.

(iii) Several bands and inter-bands form a replicon-cluster.

### (3) 1977-1981

(i) A common regulator for initiation may exist.

(ii) The regulator for initiation and termination may be different.

(iii) DNA replication on polytene chromosome follows certain patterns divisible into early, middle and terminal patterns of an S-phase. This has been shown by FudR synchronization *in vivo*.

## (III) Miscellaneous Studies

Significant contributions have also been made by Mukherjee in the fields of (i) Recombination in male, (ii) fractional mutations and repair, (iii) developmental genetics of biological patterns, (iv)

exogenous DNA incorporation, (v) hormones' role in replication and maintenance, (vi) RNA-dependent DNA polymerase, and (vii) autoradiography as a technique.

## Selected Publications

### Dosage Compensation

1. *Nature, Lond*, **207** (1965) 785-86.
2. *Nucleus*, **9** (1966) 83-96.
3. *Genet Res, Camb.*, **14** (1969) 137-50.
4. *J Cell Biol*, **47** (1970) 18-33.
5. *Genetic Res, Camb*, **15** (1970) 301-7.
6. *Proc zool Soc*, **25** (1972) 1-9.
7. *Chromosoma*, **36** (1971) 46-59.
8. *Cell Differentiation*, **2** (1973) 1-19.
9. *Nucleus*, **16** (1973) 130-46.
10. *Nucleus*, **17** (1974) 183-99.
11. *J Microsc*, **106** (1975) 199-208.
12. *Indian J exp Biol*, **13** (1975) 452-59.
13. *Chromosoma*, **53** (1975) 91-106.
14. *Chromosoma*, **54** (1976) 117-25.
15. *J Cell Biol*, **75** (1977) 168-80.
16. *Proc Int Cong Genet, USSR*, **C8** (1978) 194.
17. *Indian J exp Biol*, **16** (1978) 1027-31.
18. *Indian J exp Biol*, **17** (1979) 1374-76.
19. *Indian J exp Biol*, **18** (1980) 574-75.
20. *Genet Res, Camb*, **38** (1981) 103-13.
21. *Chromosoma*, **82** (1981) 405-14.
22. *Chromosoma*, **82** (1981) 515-23.
23. *J Cell Sci*, **47** (1981) 295-309.

### DNA Replication

1. *J Cell Biol*, **47** (1970) 18-33.
2. *Cell Differentiation*, **2** (1973) 1-19.
3. *Indian J exp Biol*, **13** (1975) 452-59.
4. *Chromosoma*, **54** (1976) 117-25.
5. *J Cell Biol*, **75** (1977) 168-80.
6. *Development and Neurobiology of Drosophila*, 1980, 57-83.

## M K K Pillai

The main area of specialization of Pillai has been insect toxicology and physiology. His significant contributions are discussed below in brief.

### (1) Insecticide Resistance in Insects

The physiological and genetical mechanisms of DDT resistance in the yellow-fever mosquito, *Aedes aegypti*, were elucidated. DDT resistance in *Ae. aegypti* was found to be due to a single gene present on chromosome 2 responsible for the production of the detoxifying enzyme DDT-ase, while mixture resistance (to DDT and Warf-antiresistant) had contributions from chromosomes 2 and 3. A novel idea of modifying DDT molecule to render it less open to detoxication was demonstrated as a counter-measure against DDT-resistant *Ae. aegypti*. These studies led to the discovery of Deutero-DDT, which was found to be highly toxic to DDT resistant strains of *Ae. aegypti*. It proved to be less vulnerable to dehydrochlorination by DDT-ase. The enzyme in mosquitoes was found to be different from that in houseflies in its substrate specificity. The use of a synergist as a counter-measure against DDT resistance in *Ae. aegypti* was not found to be desirable, as the mosquitoes developed resistance to the mixture in a few generations of selection. Seven Indian strains of *Ae. aegypti* on

selection with various organophosphorus insecticides for 20 generations did not develop resistance. However, in similar selection studies, two Indian strains of the urban malaria vector, *Anopheles stephensi*, developed moderate to high levels of resistance to different OP compounds. OP resistance in *An. stephensi* was found to be associated neither with non-specific esterases nor with the insensitivity of cholinesterase. It appears to be due to microsomal detoxication of OP compounds by the resistant strains. The insecticidal efficacy and repellent effect of a synthetic pyrethroid, deltamethrin, in four species of mosquitoes and the speed of selection for resistance to this insecticide by anopheline and culicine mosquitoes are being investigated currently.

### (2) Chemosterilization of Mosquitoes

The sterilization potential of variously substituted aziridinyl compounds, phosphorus amides and s-triazines, in mosquitoes was investigated to establish the structure-activity relationship. Among them, phosphoramides were found to be safe and potent chemosterilants for mosquitoes. These compounds were rapidly metabolized by the mosquitoes. The chemosterilized male mosquitoes were found to be as competitive as the normal males in their biological fitness.



The cytogenetic basis of chemically-induced sterility was elucidated in mosquitoes. Chemosterilization of *Earias fabia*, a serious pest of cotton, using Thiotepa was found to be a promising method for its control. For the first time, the involvement of the male accessory gland substance of mosquitoes in the regulation of blood intake by the female mosquitoes and in the fertility of eggs in mosquitoes was demonstrated.

### (3) Environmental Pollution by Pesticides

The extent of environmental pollution by organochlorine insecticides in Delhi area has been monitored during the last several years. A survey of 182 blood samples from people in Delhi area in 1975 established the presence of high levels of residues of DDT and its metabolites as compared to those in many developed countries. Soil and earthworm samples collected from many localities in Delhi in 1974 also showed significant levels of DDT residues in soil with very high biomagnification in earthworms, especially from areas located near the DDT factory. A two-year survey of Jamuna river at four different sites in Delhi during 1976-78 established moderate levels of DDT residues in bottom samples and in a few non-target organisms. The total DDT residue in water was less than the recommended level, but it exceeded the recommended level in certain species of fishes. Regular monitoring of organochlorine residues in air and rain water during 1980-82 showed high levels of DDT and HCH residues in certain parts of the city. Biomagnification, recycling, metabolism and elimination of organochlorine insecticides in many aquatic non-target organisms were

investigated with a view to establishing the overall impact of pesticide residues on the aquatic ecosystem. Preliminary studies have shown that DDT and HCH dissipate rapidly from the soil in tropical and subtropical countries as compared to temperate countries. Studies are in progress to precisely quantify the half-life of DDT and HCH in Indian soil under field conditions.

### Selected Publications

1. Pillai M K K, Abedi Z H & Brown A W A, WARF antiresistant compounds as synergists against DDT-resistant *Aedes aegypti*, *Mosquito News*, **23** (1963) 112-17.
2. Pillai M K K, Hennessy D J & Brown A W A, Deuterated analogues as remedial insecticides against DDT-resistant *Aedes aegypti*, *Mosquito News*, **23** (1963) 118-25.
3. Pillai M K K & Brown A W A, Physiological and genetical studies on resistance to DDT substitutes in *Aedes aegypti*, *J econ Entomol*, **58** (1965) 255-66.
4. Pillai M K K & Grover K K, Chemosterilization of *Culex pipiens fatigans* Wiedemann by exposure of aquatic stages. I. Sterilization potential of certain aziridiny compounds, *Bull Wld Hlth Org*, **41** (1969) 915-28.
5. Grover K K & Pillai M K K, Chemosterilization of *Culex pipiens fatigans* Wiedemann by exposure of aquatic stages. II. Sterilization potential of certain phosphoramides and s-triazines, *Bull Wld Hlth Org*, **41** (1969) 929-36.
6. Grover K K & Pillai M K K, The mating ability of males of *Culex pipiens fatigans* Wiedemann with apholate or tepa, *Bull Wld Hlth Org*, **42** (1970) 807-15.
7. Madhukar B V R & Pillai M K K, Development of organophosphorus resistance in Indian strains of *Aedes aegypti* (L); *Bull Wld Hlth Org*, **43** (1970) 735-45.
8. Pillai M K K, Chemosterilants for mosquitoes, in *Insecticides*, Vol 1, edited by A S Tahori (Gordon and Biard Publishers, London) 1972, 483-94.
9. Pillai M K K, Bhasin V K, Terry P H & Borkovec A B, Structure-activity relationships of phosphorus amides in *Aedes aegypti* and *Culex*

- pipiens fatigans* (Diptera: Culicidae), *J med Ent*, **12** (1975) 497-502.
10. Adlakha Vimla & Pillai M K K, Involvement of male accessory gland substance in the fertility of mosquitoes, *J Insect Physiol*, **21** (1975) 1453-57.
11. Yadav D V, Pillai M K K & Agarwal H C, Uptake and metabolism of DDT and lindane by the earthworm, *Pheretima posthuma*, *Bull Environ Contam Toxicol*, **16** (1976) 541-45.
12. Adlakha Vimla & Pillai M K K, Role of male accessory gland substance in the regulation of blood intake by mosquitoes, *J Insect Physiol*, **22** (1976) 1441-42.
13. Yadav D V, Mittal P K, Agarwal H C & Pillai M K K, Organochlorine insecticide residues in soil and earthworms in the Delhi area, India, August-October, *Pestic Monit J*, **15** (1981) 80-85.
14. Chitra S & Pillai M K K, Role of non-specific esterases and cholinesterases in organophosphorus and carbamate resistance in *Anopheles stephensi*, *Indian J exp Biol*, **23** (1985) 576-84.
15. Agarwal H C, Mittal P K, Menon K B & Pillai M K K, DDT residues in the River Jamuna in Delhi, India, *Water, Air Soil Pollut*, **28** (1986) 89-104.



## M R N Prasad

Prasad has been active in research since 1946 and established a flourishing research group at the Department of Zoology, University of Delhi which was recognized nationally and internationally as a centre of excellence in research and research training in reproductive physiology. Twenty-six PhD's were trained, many of whom occupy senior positions in research in the field in India and USA.

### Regulation of Male Fertility

Recognizing the importance of application of basic research to problems of contraceptive development, which is a national priority in India, Prasad initiated studies in laboratory animals and non-human primates aimed at elucidating factors regulating reproduction in the male. These studies led to the postulation of a "Differential androgen threshold hypothesis" regulating the function of the epididymis and maturation of spermatozoa.

The spermatozoa released from the testis acquire characteristic patterns of motility and ability to fertilize ova during their transit through the epididymis, a highly coiled duct associated with the testis. The functional integrity of the epididymis and maintenance of an internal milieu conducive for sperm maturation are dependent on higher levels of the male hormone testosterone (and its metabolite

dihydrotestosterone) than those required for the maintenance of libido/sexual potential and function of the prostate and seminal vesicles and quantity/quality of the ejaculate. Exploiting this differential androgen requirement for the maintenance of functional integrity of different parts of the male reproductive system, it was shown that microquantities of an antiandrogen (antagonist of androgen), cyproterone acetate, released through subcutaneously implanted silastic capsules inhibited maturation of spermatozoa in the epididymis by selective deprivation of androgens to the epididymis. Functional sterility was thus induced in rats without any effects on libido or accessory gland function.

Based on these studies, a new approach to control of fertility in the male was proposed in 1971.

The World Health Organization's Special Programme of Research and Research Training in Human Reproduction initiated clinical trials to evaluate the feasibility of regulation of fertility in the human male by this approach using the antiandrogen, cyproterone acetate.

These clinical trials carried out in the Federal Republic of Germany, Denmark, Hong Kong and India showed that 5-10 mg day of cyproterone acetate administered orally resulted in severe

---

Formerly, Professor of Zoology, University of Delhi, Delhi-110007 and Scientist, World Health Organization, Geneva, Switzerland; Residence : 392, III Block, 2nd Cross, Koramangala Extension, Bangalore-560034.

oligospermia and decrease in motility/viability of spermatozoa without any effects on the quantity/quality of the ejaculate.

Since cyproterone acetate is an antiandrogen with progestational activity, there was a slight reduction in endogenous androgen levels and loss of libido in two subjects. The World Health Organization, therefore, recommended another series of clinical trials to evaluate the effects of a combination of cyproterone acetate and an androgen. WHO was unable to carry out these studies due to financial constraints.

The National Institute of Health and Family Welfare in Delhi carried out a clinical trial with the combination of cyproterone acetate and androgen and showed that azoospermia was induced in all the six treated subjects without loss of libido. Encouraged by these results, the Indian Council of Medical Research initiated an extended series of clinical trials in 1984. Should these additional trials yield satisfactory results, it would lead to the availability of the long awaited and much needed orally administered oral pill for fertility regulation in the human male in 6-7 years. These developments owe their origin to the basic research carried out by Prasad.

Research on hormonal regulation of spermatogenesis has led to the demonstration of the role of FSH in the initiation and maintenance of spermatogenesis in rats and primates and opens the way for the use of FSH antagonists to selectively inhibit spermatogenesis.

In recognition of these activities, the International Planned Parenthood

Federation invited Prasad to organize a workshop in India on control of fertility in the male. The workshop was held in 1974 and its proceedings were published as a supplement to the *Journal of Reproduction and Fertility*.

### **Hormonal Regulation of Implantation**

The unique dependence of the hamster on progesterone alone for ovum implantation in the hamster, which was demonstrated in 1958, has led to its use as a suitable animal model for the bioassay of progestins and for the screening of agents involved in interference with implantation.

The role of estrogen in initiating metabolic changes as a prerequisite to implantation has been studied in the rat. A number of antiestrogens have been studied in the rat to evaluate their potential for use as postcoital contraceptives. The mechanism of action of several of these compounds indicates the possibility of their use at selected times during the peri- and post-ovulatory period for inhibition of fertility. WHO is pursuing this approach and is evaluating the contraceptive efficacy of selected antiestrogens in primates.

### **Research Training**

Research training is an integral component of research. Prasad organized for the first time in India an integrated training programme in reproductive physiology from 1963 to 1972 at the Department of Zoology in the Delhi University. These programmes of 3-6 months' duration were attended by more than 120 scientists from almost all institutions in India interested in research in reproductive physiology. The faculty for these training programmes were drawn



from leading institutions in India and abroad.

### **Use of Non-Human Primates in Biomedical Research**

Prasad has spearheaded efforts in strengthening facilities for research involving the use of non-human primates in biomedical research in India. Even though India had been exporting rhesus monkeys for biomedical research and drug development throughout the world for over four decades, appropriate facilities were not available to Indian scientists to use primates for research within the country. Prasad was the Chairman of a Subcommittee of the Planning Commission which drew up in 1971, a proposal for the establishment and strengthening of primate facilities in India and conservation of primates in the wild. These have led to the strengthening of primate facilities in Lucknow, Delhi, Bombay and Bangalore. In collaboration with T C Anand Kumar, Prasad organized under the auspices of the Indian National Science Academy, an international conference on the use of non-human primates for biomedical research for the first time in India in 1975. This conference drew up guidelines for judicious use of non-human primates for biomedical research and proposed a national ecological study of the primates in the wild and evolution of a plan for the conservation of primates which are a precious national resource. The Government of India initiated a national ecological study and imposed total ban on the export of monkeys from India in 1976. These measures have led to the stabilization of the primate populations in the wild. The International Primatological Society welcomed the guidelines for use of

non-human primates in biomedical research.

### **Comparative Endocrinology**

Prasad has been in the forefront of research in comparative endocrinology in India. His involvement in this area began in 1946 with research on the five striped south Indian palm squirrel; this was extended in later years to extensive studies on the field rat *Tatera indica*, the three striped palm squirrel of north India, the giant fruit bat, the Indian house lizard and the fish. In recognition of these contributions, Prasad was elected Chairman of the international organizing committee for comparative endocrinology in 1963 to organize the fifth international symposium on comparative endocrinology in India in 1967.

### **Association with Indian Institutions**

Prasad was actively associated with research on human reproduction and regulation of fertility in India since 1963, when the Government of India initiated support for a major research effort in this field. He was a Member of the National Coordination Committee of the Ministry of Health and Family Welfare, Government of India (1972-77); Member of the Expert Committee on Biomedical Research of the Indian Council of Medical Research (1971-1973); and Chairman of the Research Committee, Biology Division of the National Institute of Health and Family Welfare (1971-1975).

During his tenure as Scientist in the World Health Organization (1977-1983), Prasad was involved in coordination of research and research training at the All India Institute of Medical Sciences, New Delhi, Institute for Research in Reproduc-

tion, Bombay and the Indian Institute of Science, Bangalore and worked closely with the Indian Council of Medical Research and the Ministry of Health, Government of India in research on human reproduction and family planning programmes in India.

### **Association with International Institutions**

Prasad's contributions to research in reproduction have earned recognition in several international bodies. He is

Founder-Member of the International Society of Andrology, and was member of the Research Committee of the International Planned Parenthood Federation (1964-1975). Prasad organized several international meetings in India (Comparative Endocrinology Congress: 1967; Workshop on Control of Fertility in the Male (IPPF): 1974; and International Meeting on Use of Non-human Primates in Biomedical Research: 1975). Prasad was associated with the World Health Organization as a Scientist during 1977-1983 and as a Consultant in 1985.



## L S Ramaswami

My research career started with my joining the Department of Zoology, Central College, Bangalore, after graduation (BSc, old scheme) and being awarded the Nagar Manjia Udupa Gold Medal.

### Research on South Indian Tadpoles

My professor initiated me into the study of tadpoles. I became acquainted with four genera of frogs: *Rana* (common frog), *Bufo* (toad), *Microhyla* and *Uperodon* ('engystomatid' toads), and *Rhacophorus* (chunam frog). Each one of them had a distinctive tadpole and the metamorphosis also showed peculiarities.

As frog's legs were prized as a table delicacy, some workers suggested establishment of "frog farms", for earning foreign exchange. In this connection, I contributed an article to the *Economic Products of India* (Vol 3, 444) bringing out the hazards of frog-farming and the futility of a venture like that.

My study on the tadpoles and the cranial morphology of adult *Anura amphibia* (frogs) with the aid of microtome sections, a novel method of study, brought out many new findings. I published a number of papers on cranial morphology of frogs. I submitted 10 selected papers out of these (with a review thereon) to the Madras University and obtained the degree of Doctor of Science.

### Adaptive Modifications in Tadpoles Living in Fast-running Brooks

During our zoological excursions into the forests, we collected tadpoles in the waterfalls anchored to the boulders behind by a suctorial mouth. Their cartilaginous skull (chondrocranium) showed resemblance to that of the South African cystignathid *Heleophryne* (whose tadpoles sent to me from S. Africa were also studied by me). Similarities between African and Indian fauna are well known. Another tadpole (*Rana afghana*) which shows a different type of attachment to rocks in fast-running brooks was also described by me. I collected another tadpole (of frog *Philautus*) from the forests of Karnataka and described its larval skull peculiarities. My studies on the *Anuran chondrocranium* attracted the attention of Prof. E S Goodrich, FRS (Linacre Professor of Zoology at Oxford), who was later to be one of my DSc examiners.

### Induction of Spawning in Frog and Food-Fish of India

The thrilling part of my frog developmental studies was to induce spawning in gravid females and then fertilize the eggs with sperm from male (with testes homogenized in Holtfreter saline solution). We discovered that during the breeding season (monsoon rains), the

female pituitary gland secretes a number of hormones and these work on the gonads and ripen them. The cytochemistry of the pituitary gland was studied and described by us. The epoch-making basic study led us on to use fish as our raw material. The catfish *Heteropneustes fossilis* (an air-breather) proved to be an excellent experimental animal. After injecting a small dose of pituitary (taken from *Heteropneustes* or *Clarias*) and stripping the injected fish after 8-10 h, the eggs were fertilized with sperm. The developmental stages were studied step by step. Hybridization experiments using *Heteropneustes* and *Clarias* proved fruitless, as the developing fry suffered from a genetic imbalance called 'haploid syndrome'. This success led us on to use food-fish (Labeo, etc.) and our spawning experiments were very successful. Hatching fry from food-fish and stocking ponds with pure seed became a reality.

I presented these results in my Presidential Address to the Zoology and Entomology Section of the Indian Science Congress, Cuttack, 1962<sup>4</sup>. At the General and Comparative Endocrinology Symposium at Oiso, Japan, in 1962, I presented a paper on the anuran and piscine pituitary gland cytology based on my histochemical studies<sup>5</sup>.

### Apoda Amphibia (Limbless Frogs)

While I was engaged in chondrocranial and experimental fish studies, another group of Amphibia, even more fascinating than the frogs, attracted my attention, viz. *Apoda amphibia* (limbless frogs). They look like snakes (also called gymnophiona), but are extremely harmless, so that one could handle them with impunity. Zoogeographically, they are

restricted to the tropics and lead a subterranean life. One has to dig by the side of flowing streams and if one is lucky, one may land one or two. They venture out during the nights for feeding (earthworms and termites as seen from the stomach content). Their habitats in India are the western and eastern ghats, Meghalaya, Sikkim, Assam, etc. The Sarasin brothers came all the way from Switzerland to collect and study the common genus *Ichthyophis* in Sri Lanka. We collected the same genus in Kotgehar, Sringeri, etc. This Ishmaelite has set evolutionary biologists thinking: whether the Apods are primarily simple (according to Marcus, Germany) or secondarily simplified (Goodrich, Oxford) is the bone of contention. Since they live under the soil, they have retained their primitive characters (e.g., scales on the skin) according to Marcus. This is a difficult question to solve.

These cryptic animals lay a clutch of eggs by water margins during the rainy season and the embryos develop inside the eggs. We have collected egg clutches. The embryos lose their external gills and then they hatch; the larvae enter the water and after some time they start burrowing in the soil. I have published more than 12 papers on this group.

The following are the major findings of this study. The limbless frogs lead a subterranean life, but venture out during nights for feeding. They breed during the rainy season. One species of Apoda has been named after me: *Gegeneophis ramaswamii* Taylor. Taylor is an American authority on this group of Amphibia. I was the first to record the occurrence of Apoda from the Eastern ghats.



## Fish Skulls

Coming to know about my interesting studies on larval skulls, Dr S L Hora (ex-Director of the Zoological Survey of India, Calcutta) suggested that I should explore the fish-skull, particularly to see if it throws any light on the phylogeny of Cyprinidae in which he was greatly interested. I was awarded a junior fellowship of the Indian National Science Academy (then the National Institute of Sciences of India) and I started studying fish-skulls at the Department of Zoology, Central College, Bangalore. I published more than 12 papers during the tenure of this fellowship.

## Primate Studies

While at Jaipur, having built a flourishing department of zoology from scratch, I was amazed at the monkey raw material available there. The Hanuman langur (*Presbytis entellus*) could be had in numbers and a local specimen collector was ready to supply and stock our animal house. The Ford Foundation had generously funded my project and the monkey house started teeming with life—male and female langurs. It was a caged colony. Our studies have provided some very important results which have been published from time to time. The important findings are given below.

(1) The cervical canal in the female langur is straight, so that intrauterine devices could be inserted without difficulty into the cervical canal unlike in rhesus and bonnet monkeys where laparotomy alone could help. At the opening session of the seventh International Primatological Conference at Bangalore in 1979, the Director General, ICMR, referred to this important finding of ours. In addition, the fallopian tubes of the Hanuman langur are

also straight. In both these features, the langur resembles the human female.

(2) The menstrual cycle is of 22 days' duration and peculiarly, ovulation takes place on day 9-10 of the cycle. I presented a paper on the menstrual cycle in the Hanuman langur at the International Conference on Primates at Cambridge, England, 1976, which was well received.

(3) Oestrone is the major urinary oestrogen excreted by the animal. Oestrogen excretion reaches a peak at the time of ovulation.

(4) According to our finding, sialic acid is not androgen dependent in this monkey.

(5) Spermatogenesis continues right through the year and the male monkey is reproductively active throughout the year.

(6) The percentage testis weight resembles that in the human; in the macaques, it is very much more than in the human.

In recognition of the work done by me on a non-human monkey, the Indian National Science Academy awarded me the prestigious Sunder Lal Hora Gold Medal for 1975. I delivered the Hora Lecture at the Delhi University.

The following were the reasons for the selection of the frog *Rana cyanophlyctis*, the fish *Heteropneustes fossilis* and the monkey *Presbytis entellus*:

(1) The frog *R. cyanophlyctis* is available throughout India and is gravid throughout the year. Being small in size, it is easy to handle unlike the green frog and the bull frog. Induction of spawning is easy in this species and in institutions where experimental embryology is taught, this frog is a must. We have published the stage by stage development of this frog.

(2) We were the first to use the freshwater catfish *Heteropneustes fossilis* (which is also used as food) for induced spawning experiments, as the animal is quite hardy. There are no scales. It is an air-breather. Hypophysectomy can be done easily. This gave us an insight into inducing spawning in food-fish, a great asset for fishery biologists.

(3) The only feature that prompted us to use the Hanuman langur (*Presbytis entellus*) was its availability. It stands castration and ovariectomy without much post-operational effects. Blood can be drawn from the saphenous vein easily under anaesthesia. In the north, it is available abundantly.

### Selected Publications

1. Ramaswami L S, The cranial osteology of the South Indian *Engystomatidae* (Anura), *Half yearly J Mysore Univ*, **6** (1932) 45.
2. Ramaswami L S, The vertebral column of some South Indian frogs, *Curr Sci*, **1** (1933) 306.
3. Ramaswami L S, An account of the chondrocranium of *Rana afghana* and *Megophrys* with a description of the masticatory musculature of some tadpoles, *Proc natn Inst Sci India*, **9** (1943) 43.
4. Ramaswami L S, An account of heart and associated vessels in some genera of *Apoda* (Amphibia), *Proc zool Soc, Lond*, **114** (1944) 117.
5. Ramaswami L S, The chondrocranium of two torrent dwelling anuran tadpoles, *J Morph*, **74** (1944) 347.
6. Ramaswami L S, Apodous amphibia of the Eastern ghats, South India, *Curr Sci*, **16** (1947) 8.
7. Ramaswami L S, Skeleton of Cyprinoid fishes in relation to phylogenetic studies: I. The skull and Weberian apparatus of Cyprininae (Cyprinidae), *Acta Zool*, **36** (1955) 199.
8. Ramaswami L S & Sundararaj B I, Induced spawning in the Indian catfish *Heteropneustes* with pituitary injections, *Acta Anat*, **31** (1957) 551.
9. Ramaswami L S & Lakshman A B, The skipper frog as a suitable embryological animal and an account of the action of mammalian hormones on spawning the same, *Proc natn Inst Sci India*, **25** (1959) 68.
10. Ramaswami L S, Endocrinology of reproduction in fish and frog, *Gen Comp Endocrinol* (1) (1960) 206.
11. David G F X & Ramaswami L S, Studies on menstrual cycle and other related phenomena in the langur (*Presbytis entellus entellus*), *Fol Primat*, **11** (1969) 300.
12. David G F X & Ramaswami L S, Reproductive system of the North Indian langur (*Presbytis entellus entellus* Duf.), *J Morph*, **135** (1971) 99.
13. Ramaswami L S, Vertebrate neurosecretion—A review, *Proc Indian natn Sci Acad*, **B46** (1980).
14. Ramaswami L S, Kurup G V & Gadgil B A, Some aspects of the reproductive biology of the lion-tailed macaque *Macaca silenus* (Linn.) A zoo study, *J Bombay natu Hist Soc*, **79** (1982) 324.
15. Ramaswami L S, The anatomy, histology and biochemistry of the mammalian testis, *Proc Indian natn Sci Acad*, (1983) 1-93.



# S R V Rao

## (1) Studies on X Chromosome Regulation

One of the major interests in molecular biology centres around the problem of X chromosome regulation. This regulation, at least in *Drosophila* and mammals, brings about the equalization of the X-linked gene products between sexes in spite of X chromosome aneuploidy. Since all the male heterogametic organisms display X-chromosome aneuploidy (XO/XY male; XX female), it is reasonable to assume that all such organisms would exhibit dosage compensatory mechanism of either *Drosophila* type (male X hyperactivation) or mammalian type (female X inactivation).

Therefore, studies were undertaken to inquire if any of the above-mentioned mechanisms operate in other groups of animals as well. Two Orthopteran insects, *Gryllotalpa fossor* (mole cricket) and *Acheta domesticus* (house cricket), both belonging to XO male, XX female sex chromosome mechanism, were chosen. In the absence of any genetic information, we examined the problem by studying the functional status of the X chromosome through (a) DNA replication by <sup>3</sup>H-thymidine autoradiography and 5-bromodeoxyuridine acridine orange (5-BrdU/AO) fluorescence microscopy, (b) <sup>3</sup>H-uridine induced aberrations to assess

the transcriptional activity, and (c) isoenzyme (G6PD) studies.

These studies have enabled us to discover both X-chromosome inactivation mechanism (in *Gryllotalpa*) and male X hyperactivation mechanism (in *Acheta*), respectively. From these studies, we believe that the X regulatory mechanism may be of widespread occurrence.

Our preliminary studies on *Gryllotalpa* indicate that the X chromosome inactivation mechanism is probably due to DNA modification by methylation. It would be of great importance to confirm this through other molecular approaches, viz., cloning the G6PD and other X linked genes and studying the methylation patterns during active and inactive states.

## (2) Heterochromatin Polymorphism and Fragile Sites in *Nesokia indica*

The karyotype of *Nesokia* consists of 42 chromosomes, X and Y being the largest and the second largest metacentrics respectively, in the complement. Extensive work over 15 years covering about 900 animals of both sexes revealed the occurrence of a variety of X and Y chromosomes. Remarkably, the observed polymorphism is confined exclusively to the heterochromatin of X and Y. Of interest is the fact that only animals with the entire amount of

heterochromatin predominate ( $> 80\%$ ). Implicit in this is the fact that there could be selection against those lacking or endowed with incomplete amount of heterochromatin.

In order to test if heterochromatin in any way influences reproductive performance, laboratory breeding experiments were carried out with animals with various polymorphic X and Y chromosomes. The results suggested that heterochromatin does play "some" role in the population dynamics of *Nesokia*. The studies also indicated that the heterochromatin in *Nesokia* may not be composed of only redundant sequences, which could be dispensed with and may contain some "cryptic unique sequences".

It is known that in man, the X linked fragile sites seem to influence the reproductive behaviour of the affected males (hemizygotes) and such males are mentally retarded. However, heterozygous female carriers are normal.

A search for similar fragile sites led to their discovery on the X chromosome of *Nesokia*. What is most significant is that the fragile site/s is/are located in the heterochromatin. Males hemizygous for this condition fail to show any reproductive performance. It is equally

significant that, unlike humans, females homozygous for fragile sites are also reproductively ineffective. We believe our work to be the first demonstration of fragile sites in non-humans. Further work is planned to understand the nature of fragile sites.

### Selected Publications

1. Arora P & Rao S R V, Insect sex chromosomes V. 3H-Uridine induced aberrations in the X chromosomes of tetraploid spermatogonia from *Gryllotalpa fossor*, *Chromosoma, Berl*, **77** (1980) 373-78.
2. Bhattacharya M, Ali Sher & Rao S R V, Insect sex chromosomes. VIII. Identification of active/inactive X chromosomes in *Gryllotalpa fossor*, *Exp Cell Res*, **144** (1983) 228-31.
3. Rao S R V & Arora P, Insect sex chromosomes. III. Differential susceptibility of homologous X chromosomes of *Gryllotalpa fossor* to 3H-uridine induced aberrations, *Chromosoma, Berl*, **74** (1979) 241-52.
4. Rao S R V, & Ali S, Insect sex chromosomes. IV. A presumptive hyperactive activation of the male X chromosome in *Acheta domesticus* (L), *Chromosoma, Berl*, **86** (1982) 325-39.
5. Rao S R V, Vasantha K, Thelma B K, Juyal R C & Jhanwar S C, Heterochromatin variation sex chromosome polymorphism in *Nesokia indica*: A population study, *Cytogenet Cell Genet*, **35** (1983) 233-37.
6. Rao S R V & Bhattacharya M, Insect sex chromosomes. IV. X chromosome linkage of glucose 6-phosphate dehydrogenase (G6PD) locus in *Gryllotalpa fossor* (Orthoptera), *Heredity*, **53** (1984) 235-38.



## M L Roonwal

The main disciplines that I have been able to cover during the last half century of research activity (since 1930) are: embryology, entomology, taxonomy, ecology and primate behaviour.

### (1) Embryology

I began with embryology and published, in 1936 and 1937, two monographs<sup>1,2</sup> on the embryology of the African migratory locust. The first one included a new theory of multiphased gastrulation in insects, according to which the process of gastrulation (the early, post-cleavage rearrangement, whether by invagination or by epiboly, of two of the three primary germ layers, mesoderm and endoderm) occurs in not one but several (multiphased) spurts of activity. The theory was later extended to cover other arthropods<sup>3</sup>.

### (2) Entomology

The main aspects covered have been agricultural and forest entomology, especially locusts and termites.

*Locusts* : The principal findings include new phase characters, especially eye-stripes, and their bearing on the understanding of population dynamics and behaviour<sup>4,5</sup>. They also led to the discovery<sup>6</sup> of a new evolutionary phenomenon, viz., a sharp increase in

intraspecific variation in minimum populations.

*Termites* : The main aspects studied during the last 30 years (since the early 1950's) were ecology, morphology, taxonomy and control<sup>7-12</sup>. The termite faunas of several parts of India as well as other countries (Indonesia and Brazil) have been investigated, and several new species and a new family (Indotermitidae) described. The morphological studies conducted include detailed and critical examination of the abdomen, the genitalia and the accessory reproductive structures<sup>11</sup>. A remarkably dense and unique pattern of microsculpturing on termite wings (density up to 13000/mm<sup>2</sup>, with a million or more elements on a single wing) has been discovered, and the results published, during the last 15 years, in a series of 13 papers, the final one in 1983<sup>10</sup>. Scanning electron microscope studies of these microstructures are now under way<sup>12</sup>.

### (3) Ecology and Primate Behaviour

The early work on mammals<sup>13</sup> concerned the ecology and systematics of mammals, especially rodents, of the Assam-Burma region. Later work has been on primates<sup>14-16</sup>. Apart from the book on the *Primates of South Asia*<sup>14</sup>, the principal discoveries are concerned with intraspecific variations in tail form and

---

Formerly, Director, Zoological Survey of India and Vice-Chancellor, University of Jodhpur, Jodhpur;  
Residence : 'Roonwal Bhawan', 2/1, Polo Ground No 2, Jodhpur-342006.

carriage and their evolutionary and biological significance. In the common Indian langur (*Presbytis entellus*), for example, two main styles of tail carriage have been found, viz., a Northern Style (North India down to the Vindhyas) with the tail looped forward, and a Southern Style (Peninsular India and Sri Lanka) with the tail looped backward in S-shape<sup>15,16</sup>

I have edited four books<sup>17-20</sup>.

#### (4) Future Work

For so vast a subject as the animal world, it is difficult to suggest future lines of work. The best advice would be: every one according to his choice, training and the available facilities, and do not ask for the impossible. Research problems abound all around us, especially in the field—on biology, taxonomy, ecology and behaviour—and these aspects do not require elaborate or costly equipment, beyond a few simple instruments, a good pair of observant eyes and a pencil and a notebook. Some of our commonest animals are still poorly known, and the faunas of vast tracts of the country remain to be investigated. The most important criteria for success are the will to work and to burn midnight oil, if necessary.

#### Selected Publications

1. Roonwal M L, Studies on the embryology of the African migratory locust, *Locusta migratoria migratorioides* R and F-I. The early development, with a new theory of multiphased gastrulation among insects, *Phil Trans R Soc*, **B226** (1936) 391-421.
2. Roonwal M L, Studies on the embryology of the African migratory locust, *Locusta migratoria migratorioides* R and F-II. Organogeny, *Phil Trans R Soc*, **B227** (1937) 174-244.
3. Roonwal M L, Amplification of the theory of multiphased gastrulation among insects, and its applicability to some other arthropods, *Trans natn Inst Sci India*, **2**(1) (1939), 1-37.
4. Roonwal M L, New hypotheses for the prediction of the swarming of the desert locust, *Bull ent Res, Lond*, **35** (1945) 391-93.
5. Roonwal M L, Variation and structure of the eyes in the desert locust, *Schistocerca gregaria* (Forsk), *Proc R Soc*, **B134** (1947) 245-72.
6. Roonwal M L, On a new evolutionary phenomenon: The sharp increase of intraspecific variation in minimum populations, as evidenced by the desert locust, *Rec Indian Mus*, **51**(4) (1954) 481-525.
7. Roonwal M L & Chhotani O B, Termite fauna of the Assam Region, eastern India, *Proc natn Inst Sci India*, **B28** (1962) 281-406.
8. Roonwal M L, Field ecology and ecobiogeography of Rajasthan termites : A study in desert environment, *Zool Jahrb (Syst)*, *Berl*, **103** (1976) 455-504.
9. Roonwal M L, Termite life and termite control in tropical South Asia (Scientific Publishers, Jodhpur) 1979, pp 177.
10. Roonwal M L, Evolution and systematic significance of wing microsculpturing in termites. XIII. Order Isoptera, *Proc Indian natn Sci Acad*, **B49** (1983) 359-91.
11. Roonwal M L, The abdomen, genitalia and accessory structures in termites (Isoptera), *Mem ent Soc India*, **9** (1984) 1-69.
12. Roonwal M L, Electron microscope studies of wing microsculpturing in termites (Isoptera). I. Genera *Heterotermes* and *Microtermes*, *Proc Indian natn Sci Acad*, **B51**(1) (1985) 6-14.
13. Roonwal M L, Systematics, ecology and bionomics of mammals studied in connection with tsutsugamushi disease (scrub typhus) in the Assam-Burma War Theatre, during 1945, *Trans natn Inst Sci India*, **3**(2) (1949) 67-122.
14. Roonwal M L & Mohnot S M, *Primates of South Asia : Ecology, sociobiology and behaviour* (Cambridge Mass/Harvard University Press) 1977, pp 421.
15. Roonwal M L, Field study of geographical, subspecific and clinal variations in tail carriage in the Hanuman Langur, *Presbytis entellus* (Primates), in South Asia, *Zool Anz Jena*, **202** (1979) 235-55.
16. Roonwal M L, Tail form and carriage in Asian and other primates, and their behavioral and evolutionary significance, in *Current Primate Researches*, edited by M L Roonwal, S M Mohnot and N S Rathore (Zoology Department, Jodhpur University), 1984, 93-151.



17. *Recent advances in zoology in India*, edited by M L Roonwal (Government of India, New Delhi) 1963, pp 300.
18. *Termite problems in India*, edited by M L Roonwal (CSIR, New Delhi) 1972, pp 81.
19. The natural resosurces of Rajasthan, Vols 1 and 2, edited by M L Roonwal (Jodhpur University, Jodhpur) 1977, pp 1211.
20. *Current primate researches*, edited by M L Roonwal, S M Mohnot & N S Rathore (Zoology Department, Jodhpur University, Jodhpur), 1984, pp 627.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

## Salim Ali

In India, ornithology has been, and by and large still is, the Cinderella of the biological sciences. It receives perhaps the lowest priority in the formal zoological curriculum of most of our universities, and the teaching practically ends with the laboratory dissection of a sparrow or pigeon and a textbook smattering of their morphology and physiology. Perhaps my main contribution to bird study has been in spreading an awareness about birds among the Indian public and in weaning away (at least in part) the bias of “pukka” scientists against ‘bird watching’ as a meaningful exercise. Until not so long ago, bird watching, which is in fact the first step towards scientific ornithology, was looked upon even in western countries as a wasteful way of killing time by the idle rich, and of no scientific value; only morphology, physiology and taxonomy were considered ‘scientific ornithology’. Ecology and ethology were words one hardly ever heard. In India, even amateur bird watching was confined almost entirely to a few foreigners, chiefly British civil and military officials stationed in various parts of the country, often as an antidote to boredom. The major interest of most such persons was to collect and preserve large numbers of skins and eggs of birds for the British and other museums in their homeland in the hope of discovering some undescribed novelty among them. Some of these amateurs, however, developed into

discerning and competent field naturalists who made seminal contributions to bird study and actually laid the foundation of scientific ornithology in the subcontinent.

My own interest all along has been in the *living* bird in its natural environment—its ecology. Therefore, the dry-as-dust academic and laboratory knowledge imbibed from the formal zoology curriculum was frustrating to me, though it did provide the basic scientific background. Meaningful guidance and direction for the type of study I wished to pursue was unavailable at any Indian university. It was not until much later, 1929-30, while on study leave in Germany, that it came. I then had the good fortune to be working in the Berlin University Zoological Museum with savants like Prof. Erwin Stresemann—the doyen of European ornithology—and Prof. Oskar Heinroth, rightly regarded as the ‘Father of Ethology’—the study of habits and behaviour in relation to habitats. Since my return to India, my ornithological studies—chiefly field surveys of birdlife in remote and unworked parts of the country—have all been heavily ecology-slanted. The departure from the old style dry systematic reporting on the specimens collected has been welcomed in ornithological circles here and abroad. In the period between the two world wars, and more particularly after the end of the



second, the world emphasis has refreshingly been shifting from the laboratory to the field.

One of my earliest and more satisfying studies, which evoked flattering reviews in frontline ornithological journals in UK, USA and Germany, was on the nesting habits of the Baya weaver bird, published in *JBNHS* (Vol. 34) in 1931<sup>1</sup>. It presents a new understanding of the bird's breeding biology, the erroneous interpretation of which had become traditional. The findings have been tested and retested by myself and other ornithologists in India, and on allied species of weaver birds in Africa, and the new version has now received general acceptance. From the 100-hour study, the Baya, a colonial nester, emerges as a polygynous species having a mating system all its own. Nest building is the entire responsibility of the male. When the nests are half completed, a prospecting party of ready-to-breed females visits the 'housing colony'. The individual hens hop from nest to nest deliberately scrutinizing its workmanship. In the process, several nests are often rejected. If a female approves a nest, she takes possession of it and in turn is accepted by the builder as his wife No. 1. She lays eggs within, incubates them, and in due course raises the chicks. All this is solely her business. Having settled the female within, the cock completes nest No. 1 and proceeds to build a second nest nearby, and in due sequence the whole process is repeated. In this way, a single male Baya may acquire 3 or 4 wives and as many families, all within the same short breeding season—the monsoon.

It may seem redundant, but I give this summary abstract of the Baya paper as I know there still are many non-biologist

scientists—even some FNAs amongst them—who are unfamiliar with the type of work that 'Field Ornithology' involves and have reservations about considering 'bird watching' a serious scientific discipline. It is good, therefore, to be reminded that the two recent Nobel Laureates in biology, Konrad Lorenz and Niko Tinbergen, also started their careers as amateur bird watchers.

My other special areas of interest and research have mainly been Economic Ornithology, Bird Migration and Conservation. The importance of birds in a country like ours, which leans so heavily on agriculture and forestry, has not been sufficiently appreciated. A review paper<sup>2</sup> on "Economic Ornithology in India" which I published in '*Current Science*', as long ago as January 1936, failed to impress the government of the day, but with continued 'plugging', the subject is at last beginning to show feeble signs of official stirring. Two of my more significant papers<sup>3,4</sup> bearing on Economic Ornithology are regarded as pioneering contributions on Ornithophily, meaning the pollination of flowers through the agency of birds, a topic which has hitherto received scant attention from Indian botanists.

Except for technical reports on my regional bird surveys and expeditions, and popular 'propagandist' articles in the public media, my publications have been mostly in the form of books rather than papers in scientific journals. They are based directly upon data obtained during the field work. Of the 8 titles I have published on Indian birds, the two I consider most significant are: *The Book of Indian Birds* (BNHS, 1941)<sup>5</sup> progressively revised and expanded and now in the eleventh edition, and the comprehensive

10-volume *Handbook of the Birds of India and Pakistan* (Oxford University Press, 1968-74)<sup>6</sup> written jointly with S Dillon Ripley. The former has proved particularly rewarding, as it was the first book on Indian birds illustrated in colour and has been largely responsible for the spread of popular interest in birds and bird watching within the country.

### Selected Publications

1. Salim Ali, The nesting habits of the Baya (*Ploceus philippinus*) : A new interpretation of their domestic relations, *J Bombay natu Hist Soc*, **34** (1931) 947-64.
2. Salim Ali, Economic Ornithology in India, *Curr Sci*, **4** (January 1936) 472-78.
3. Salim Ali, The role of sunbirds and flowerpeckers in the propagation and distribution of the tree-parasite *Loranthus longiflorus* Desr in the Konkan (W India), *J Bombay natu Hist Soc*, **35** (1932) 144-49.
4. Salim Ali, Flower-birds and bird-flowers in India, *J Bombay natu Hist Soc*, **35** (1932) 573-605.
5. Salim Ali, *The book of Indian birds* (Bombay Natural History Society, Bombay) 1941, 11th edn, 1979, pp 187.
6. Salim Ali & Ripley S Dillon, *Handbook of the birds of India and Pakistan*, Vols 1-10 (Oxford University Press), 1968-74.
7. Salim Ali, Bird study in India: Its history and its importance, *Azad Memorial Lecture, Indian Council of Cultural Relations*, 1978.
8. Salim Ali, *The birds of Kutch* (Oxford University Press) 1945, pp 175.
9. Salim Ali, *Indian hill birds* (Oxford University Press) 1949, pp 188.
10. Salim Ali, *The birds of Travancore & Cochin* (Oxford University Press) 1953; 2nd edn as *Birds of Kerala*, 1969, pp 444.
11. Salim Ali, *The birds of Sikkim* (Oxford University Press) 1962, pp 414.
12. Salim Ali, *Field guide to the birds of the Eastern Himalayas* (Oxford University Press) 1977, pp 265.
13. Salim Ali & Ripley S Dillon, *A pictorial guide to the birds of the Indian subcontinent* (Bombay Natural History Society) 1983, pp 177.
14. Salim Ali & Laeeq Futehally, *Common birds* (National Book Trust, India) 1967; 5th edn, 1982, pp 117.



## A G Sathyanesan

Different aspects of the reproductive cycle of several teleostean fishes were studied in depth bringing out their correlation with the pituitary cycle. The circannual changes in the hypothalamic nuclei, pituitary, gonads, thyroid and interrenal were reported<sup>1,2</sup>. Thyroid physiology was studied extensively using inhibitors and stimulators and after radiothyroidectomy and under changing environmental conditions. Functional ectopic pronephric thyroid in a few Indian fishes was reported for the first time; study of their physiology revealed that they are normal, consistent in their occurrence and more active than the pharyngeal thyroid. Hence, in any investigation related to thyroid function in such species, thyroids of both the sites should be taken into account<sup>3</sup>. The structure and function of the interrenal and chromaffin cells of the teleosts were investigated extensively adopting various experimental techniques and their homology with the cortical and medullary cells of the higher vertebrates was confirmed<sup>4,5</sup>. Various cell types of the hypophysis responsible for secreting different trophic hormones which control the functions of the peripheral endocrine glands were identified using inhibitors and stimulators of the target glands or by extirpating them<sup>6</sup>.

Adopting a modified bulk histochemical staining procedure, the less known

complicated stereometric architectural patterns of the preoptico-neurohypophysial neuro-secretory system of several teleostean fishes were described. It has also provided additional information about the extrahypophysial neurosecretory tracts of important functional significance. After hypophysectomy, the regenerating capacity of the pituitary to form a neurohypophysis-like organ was demonstrated *in situ*<sup>7-9</sup>. This modified technique was adopted to acquire new information about the hypothalamo-neurohypophysial complex of the wall lizard *Hemidactylus flaviviridis*<sup>10</sup> and the palm squirrel *Funnambulus pennanti*<sup>11</sup>.

The morphological presence of a hypothalamo-hypophysial portal system of the tetrapodan type which was repeatedly reported to be absent in the literature was described for the first time in a few Indian teleost fishes<sup>12</sup>. This system occurs in addition to the neuro-aden interface vasculature, which is said to be equivalent to the hypothalamo-hypophysial portal system of the tetrapods.

In the chimaeroid fishes, the preoptico-neurohypophysial neurosecretory system is described in the literature as a diffused type. In one of the chimaeroid fishes, *Hydrolagus coliei*, in addition to a distinct well-defined preoptico-hypophysial neurosecretory complex, a hypothalamo-

---

Professor, Department of Zoology, Banaras Hindu University, Varanasi-221005; Residence : G, 13, Beyond Ladies Colony, Banaras Hindu University, Varanasi-221005.

hypophysial portal system was also described, which was not reported earlier<sup>13</sup>. Apart from this, rachendachhypophyse comparable to the ventral lobe of the elasmobranchs was also described in the above species. Hypothalamo-hypophysial complexes of a few primitive actinopterygians belonging to the families holostei and chondrostei were investigated in detail.

The ependymal tanocytes lining the third ventricle and the infundibular recess and their relationship with the neurosecretory neurons of the nucleus lateralis tuberis, the tract of the preoptic nucleus and the pituicytes of the neurohypophysis were firmly established in a few species of teleosts, using various parameters<sup>14,15</sup>. Pineal structure and function have been studied extensively in a few teleosts. A rare phenomenon of innervation of the third ventricular ependyma by the photosensory neurons of the pineal was also reported<sup>16</sup>. Thus, a direct relationship between the pineal, ependymal cells, hypothalamic nuclei and the hypophysis has been established. This relationship is of great importance in the physiology of neuroendocrine integration.

The phenomenon of regeneration and uncontrolled growth of the lesioned preoptico-neurohypophysial tract was reported for the first time<sup>17</sup>.

These studies revealed that the hypophysio-trophic neurohormones secreted by the hypothalamic nuclei control the hypophysial function. The biogenic monoamines pool in the hypothalamus probably modulates the release of the neurohormones. Thus, it may be possible to modify the hypophysial function through manipulation at the hypothalamic level. Further studies on

these lines will be of great academic and applied value. One aspect of this study relevant to pisciculture will be induction of spawning by manipulating the relevant monoamines at the hypothalamic level to cause a spurt in gonadotropins during the spawning phase.

The endocrine physiology of fishes was studied after exposing them to a variety of pollutants in sublethal doses comparable to the quantities present in polluted waters. The pollutants studied, and/or being investigated, are fertilizers, insecticides, fungicides, cadmium, lead and mercury. At such sublethal concentrations, the fishes apparently live normally for an indefinite period, but their thyroid and gonadal activities were inhibited. Gonads never attained maturity, liver vitellogenin synthesis was impaired, and thyroxine synthesis was blocked. Hypothalamic and pituitary secretory activity were also affected adversely. Brain catecholamines and several other biochemical components exhibited drastic changes. Interrenal function was also affected. Our evidences reveal that several pollutants in sublethal doses act through the hypothalamo-hypophysial-target glands (gonads, thyroid, interrenal) axis<sup>18-22</sup>. As small quantities of some pollutants are capable of causing marked changes in the physiology of fishes, fishes can be effective indicators of water pollution.

### Selected Publications

1. Sathyanesan A G, Histological changes in the pituitary and their correlation with the gonadal cycle in some teleosts, *Cellule*, **63** (1963) 283-89.
2. Joshi B N & Sathyanesan A G, Annual cyclical changes in the monoamine oxidase (MAO) activity in the testes of the teleost *Channa punctatus* (Bloch), *Gen Comp Endocr*, **4** (1980) 240-42.



3. Sathyanesan A G, Functional renal thyroid follicles in wild specimens of the freshwater teleost *Barbus stigma* (Cuv & Val), *Z Zellforsch*, **59** (1963) 530-34.
4. Kulkarni R S & Sathyanesan A G, Adrenal histochemistry of the freshwater teleost *Labeo rohita* (Ham), *Arch Ital Anat E Embryol*, **84** (1979) 171-81.
5. Joshi B N & Sathyanesan A G, A histochemical study on the adrenal steroidogenic cells of the freshwater teleost *Channa punctatus* (Bloch) following hydrocortisone acetate administration and hypophysectomy, *Proc Indian natn Sci Acad*, **B45** (1979) 462-68.
6. Joy K P & Sathyanesan A G, Functional cytology of the pituitary gland of the teleost *Clarias batrachus* (L), *Endokrinologie*, **73** (1973) 82-90.
7. Sathyanesan A G, An *in situ* study of the preoptico-neurohypophysial complex of the freshwater teleost *Clarias batrachus* (L), *Z Zellforsch*, **98** (1969) 202-16.
8. Sathyanesan A G, Stereometric structure of the hypothalamo-neurohypophysial complex in some Indian catfishes, *Neuroendocrinology*, **5** (1969) 10-23.
9. Sathyanesan A G, Hypothalamo-hypophysial neurosecretory system in fishes under some experimental conditions, *Gen Comp Endocr*, **2** (Suppl) (1969) 268-74.
10. Haider S & Sathyanesan A G, Hypothalamo-hypophysial neurosecretory and portal system of the Indian wall lizard *Hemidactylus flaviviridis*, *Acta Anat*, **88** (1974) 502-19.
11. Vijayan E & Sathyanesan A G, *In situ* observations on the structure of the neurosecretory system in the Indian palm squirrel *Funnambulus pennanti* (Wroughton), *Neuroendocrinology*, **7** (1971) 337-47.
12. Haider S & Sathyanesan A G, Tetrapod-like features of the hypothalamo-hypophysial vascularisation in the air-breathing teleost *Heteropneustes fossilis* (Bl), *Gen Comp Endocr*, **17** (1971) 360-70.
13. Sathyanesan A G, The hypophysis and the hypothalamo-hypophysial system in the chimaeroid fish *Hydrolagus coliei* (Lay and Bennett) with a note on their vascularisation, *J Morph*, **116** (1965) 413-50.
14. Sathyanesan A G & Joy K P, A micromorphological and histoenzymological study of the third ventricular ependyma of the teleost *Clarias batrachus* (L), *Z mikros-ant Forsch*, **92** (1978) 700-22.
15. Thomas P C & Sathyanesan A G, Micromorphology of the third ventricular ependyma and infundibular recess in the mud-eel *Amphipneus cuchia* (Ham), *J Hirnforsch*, **23** (1982) 101-7.
16. Sathyanesan A G & Sastry V K S, Pineal innervation of third ventricular ependyma in the teleost *Puntius sophore* (Ham), *J Neurol Transmis*, **53** (1981) 187-92.
17. Sathyanesan A G, Regeneration and unusual growth of the neurohypophysis and neurosecretory axons in the partly or completely hypophysectomised godfish, *Experientia*, **21** (1965) 398.
18. Ram R N & Sathyanesan A G, Effect of mercuric chloride on the reproductive cycle of the teleostean fish *Channa punctatus*, *Bull Environ Contam Toxicol*, **30** (1983) 24-27.
19. Katti S R & Sathyanesan A G, Cadmium induced inhibition of brain monoamine oxidase in the freshwater catfish *Clarias batrachus* (L), *Experientia*, **40** (1983) 576-77.
20. Katti S R & Sathyanesan A G, Changes in tissue lipid and cholesterol content in the catfish *Clarias batrachus* (L) exposed to cadmium chloride, *Bull Environ Contam Toxicol*, **32** (1984) 486-90.
21. Ram R N & Sathyanesan A G, Mercurial induced brain monoamine oxidase inhibition in the teleost *Channa punctatus* (Bloch), *Bull Environ Contam Toxicol*, **35** (1985) 620-26.
22. Ram R N & Sathyanesan A G, Inclusion bodies: Formation and degeneration of the oocytes in the fish *Channa punctatus* (Bloch) in response to ammonium sulphate treatment, *Ecotoxic Environ Safety*, **11** (1986) in press.

## V C Shah

An important contribution of Shah has been the development of culture media for the successful cultivation of amphibia cells; these are now used the world over. Shah was the first to culture frog leucocytes for chromosomes and cell cycle studies<sup>1-3</sup>.

Shah did pioneering work in studying the effects of antibiotics and antimetabolites on nuclear acid synthesis and cell cycles in tissue culture cells<sup>4-6</sup>. He was among the first few workers to show differential inhibition of ribosomal RNA in nucleoli by a certain concentration of actinomycin D<sup>4</sup> as well as to demonstrate messenger RNA synthesis in isolated chloroplasts, thus indicating the presence of transcriptionally active DNA in chloroplast<sup>7</sup>.

The finding that DNA of isolated chloroplasts can replicate *in vitro* was a significant contribution of Shah<sup>8</sup>. Using autoradiography and cytochemical methods, Shah<sup>9</sup> demonstrated the presence and replication of chloroplast DNA.

Shah was among the first few workers to show that mitomycin-C induces preferential breaks in heterochromatic regions in chromosomes<sup>10,11</sup>. He provided cytological proofs for the validity of Lyon's hypothesis<sup>12</sup>. Fluctuations in levels of vital enzymes in tissues of several vertebrates

during irradiation at very low dosage levels were noted by him<sup>13-15</sup>.

Notable among the other areas investigated by Shah are: (i) Sex-chromosomes of mammals and man<sup>16-18</sup>; and (ii) Genetic and biochemical aspects of leucoderma<sup>19</sup>.

Shah is currently involved in cytogenetic survey of congenital diseases in Gujarat state<sup>20,21</sup>.

### Selected Publications

1. Shah V C, An improved technique of preparing primary cultures of isolated cells from adult frog kidney, *Experientia*, **18** (1962) 239-43.
2. Shah V C, Comparative growth characteristics and nuclear morphology of the amphibian tissues grown *in vitro*, *Lacellule*, **64** (1963) 383-96.
3. Shah V C, Rao S R V & Sharma V K, Cell generation cycle of leucocysts cultivated *in vitro* of the Indian frog, *Nucleus*, **16** (1973) 158-61.
4. Shah V C, Autoradiographic studies of the effects of antibiotics, aminoacid analogues and nucleases on the synthesis, *Cancer Res*, **23** (1963) 1137-47.
5. Shah V C, Effects of some antibiotics on cell cycles of cultured and meristematic cells, in *Regulation of growth and differentiated function in eukaryotic cells*, edited by G P Talwar (Raven Press, New York) 1975.
6. Adhvaryu S G & Shah V C, Cell proliferation and nucleic acid proliferation treated with Rifampin, *Indian J exp Biol*, **22** (1984) 350-52.
7. Shah V C & Lyman H, Studies on DNA dependent RNA synthesis in chloroplast of *Euglena gracilis*, *J Cell Biol*, **29** (1966) 174-76.



8. Scott N S, Shah V C & Smillie R M, Synthesis of chloroplast DNA in isolated chloroplast, *J Cell Biol*, **38** (1968) 151-71.
9. Shah V C, Autoradiographic and cytochemical studies of the cytoplasmic deoxyribonucleic acid of *Euglena gracilis*, *Indian J exp Biol*, **10** (1972) 182-85.
10. Arora O P, Shah V C & Rao S R V, Studies on micronuclei induced by Mitomycin C in root cells of *Vicia faba*, *Exp Cell Res*, **56** (1969) 443-48.
11. Shah V C, Rao S R V & Arora O P, Effect of mitomycin C on the root meristem cells of *Vicia faba* L. II. Induced chromosomal aberrations in relation to the cell cycle, *Indian J exp Biol*, **10** (1972) 431-35.
12. Rao S R V, Shah V C & Seshadri C, Studies on rodent chromosomes. II. Autoradiographic study of the sex chromosome of the Indian Gerbil, *Tatera indica cuverii* (Water mouse) and its bearing on Lyon's hypothesis, *Chromosome*, **23** (1969) 309-16.
13. Rao S R V, Sharma V K & Shah V C, DNA synthesis in duplicate type sex chromosomes of the Indian house shrew *Suncus murinus* (insectivora), *Cytogenetics*, **9** (1970) 384-95.
14. Rao S R V, Rao S G A, Sharma V K & Shah V C, Differential DNA replication of the Y-chromosome in the spermatogonia of the Indian house shrew, *Suncus murinus*, *Chromosome Today*, Vol 6 (Oxford University Press), 1972, 12-16.
15. Krishnamurthy D S, Roy S & Shah V C, Pattern and frequency of association of nucleolus organizer region (NOR) chromosomes in the Hanuman Langur (p. ent. ent.) and the Bonnet monkey (*M radiata*), in *Proc Int Symp on Primates* (Jodhpur University Press) 1983, 177-99.
16. Shah V C, Bhatavdekar J M & Arvinda Babu K, Effect of low dose X-irradiation on the succinate dehydrogenase activity of guinea pig, rat and mouse tissues, *Starhlentherapie*, **152** (1976) 83-91.
17. Shah V C & Bhatavdekar J M, Radiation induced alterations in splenic phosphatases, nucleic acids and protein of mouse, *J Radiat Res*, **22** (1981) 438-42.
18. Cadhia P K & Shah V C, Effects of sub-lethal dose of  $\gamma$ -irradiation on the lactate dehydrogenase level in plasma and tissues of pigeon, *Studia biophys*, **89** (1982) 147-50.
19. Shah V C, *Genetic biochemical and cytochemical studies on Leucoderma* (Gujarat University Press, Ahmedabad) 1982.
20. Shah V C, Krishnamurthy D S, Roy Sabita, Contractor P M & Desai A B, True hermaphrodite: 46XX/46XY, clinical, cytogenetic and histopathological studies, *Indian J Pediat*, **49** (1982) 881-90.
21. Krishna Murthy D S, Roy Sabita, Contractor P M & Shah V C, Trisomy 21q : 46,XX,21s<sup>+</sup>/47,XX,+21q (q22→qter) mosaicism (de novo) in a Down syndrome child, *Indian J Pediat*, **51** (1984) 363-66.

## G P Sharma

Sharma has been actively engaged in research since 1940 when he was at Lahore (now in Pakistan). In the beginning, he had been interested in morphogenesis of such important cytoplasmic inclusions as the mitochondria and the Golgi elements in the male and female germ cells of a large variety of animals. Even movement of the aberrant sperms like those of the millipedes, ticks and spiders was fully explained by him<sup>1</sup>.

At Edinburgh, UK (1945-47), he studied the incidence of blood and meat spots in eggs and also of the miniature eggs in a pure bred stock of brown leghorn maintained at the Institute of Animal Genetics there. In tracing the cause of such defective eggs, which certainly constitute a great loss to the poultry breeders, he became interested in reproductive physiology, and became one of the pioneers in making use of even the technique of artificial insemination to improve the various breeds of poultry, sheep and cattle maintained at the Indian Veterinary Research Institute, Izatnagar and the Government Livestock Farm, Hissar<sup>2</sup>. He made use of even the indigenous products like gram, bajra and methi seeds to bring the cows into proper breeding condition<sup>3</sup>.

Later, on his rejoining the Panjab University in 1951, his interest shifted to

the study of nucleus. He and his students have been studying the morphology and behaviour of chromosomes during mitosis and meiosis in a large variety of animals right from the protozoa to mammals. These studies have enabled many problems relating to the systematic position of a number of species, genera and families to be solved<sup>4</sup>.

Sexing the day old chicks is one of the most important contributions of this group to the development of the poultry industry. The breeders are always interested to sex the chicks as early as possible, for they have to feed them differently to prepare the males for the table and the females for laying more eggs. To help the breeders in this task, this group has developed a very simple technique by which the sexes can be distinguished easily by studying the chromosomes in the dividing cells of the feather papilla. A correct diagnosis of the sex can be made within half an hour by the presence of a pair of metacentric sex chromosomes indicating the male sex and of only one such chromosome in the female sex.

The members of this group are also working on the cytogenetics of cells in human cancerous tissues and other congenital anomalies. These studies are, at present, directed to establish the chromosome patterns in the abnormal

---

Professor Emeritus (Panjab University) and Emeritus Scientist (CSIR), Department of Zoology, Panjab University, Chandigarh-160014; Residence : House No. 505, Sector 36 B, Chandigarh-160036.



cells which appear at particular sites as the tumours or have the same symptoms as the congenital anomalies. Such studies are expected to enable a better understanding of the origin and development of the various abnormalities and may help in their cure in the years to come<sup>5</sup>.

Since scientists became aware of the potential of genetical control of insect vectors, a detailed programme has been chalked out to study the various vector species of mosquitoes of the north eastern and western regions of India. As many as 20 different species have been studied in detail and their salivary chromosome maps have been produced.

To make a correct assessment of environmental pollution, the role of some of the chemicals in producing chromosomal aberrations is being studied by the members of this group.

Sharma has published about 300 papers in various national and international journals. These have been cited widely. Prof. M J D White, in his book "*Animal cytology and evolution*" (University Press, Cambridge) has referred to as many as 16 of Sharma's research papers. His work on poultry finds mention in the book "*Poultry*

*breeding*" by Morley A Jull (University of Maryland, USA). In Marshall's book "*Physiology of reproduction*", the works of Sharma find a prominent mention. The work on the spermatogenesis of the various animals carried out by Sharma has been referred to in great detail in the *International Review of Cytology* (Academic Press, New York).

### Selected Publications

1. Sharma G P, Some of the fantastic spermatozoa in the animal kingdom, Presidential Address to the Fourth All India Congress of Cytology and Genetics, in *Perspectives in cytology and genetics*, Vol 4, edited by G K Manna and U Sinha [Typographers (India) at Rashtavani Printers, Mayapuri, New Delhi] 1984, 1-8.
2. Sharma G P, Artificial insemination in cattle, *Everyday Sci*, 1(2) (1953) 119-24.
3. Sharma G P, Vali K N & Suri K R, A preliminary investigation to study the effect of feeding concentrates on the frequency of oestrous in cattle, *Res Bull East Punjab Univ*, 26 (1953) 23-32.
4. Sharma G P, Chromosomes in relation to taxonomy and phylogenetic relationship of the animals, *Presidential Address, Section of Zoology and Entomology*, 53rd Ind Sci Cong, Chandigarh, 1966, 1-12.
5. Sharma G P, Parshad R & Agnish N D, Chromosome number in some malignant human tumours, *Res Bull Punjab Univ*, 14 (1963) 99-113.

## V P Sharma

Sharma has made important contributions in several areas of mosquito biology, vector control and malariology. His early demonstration that spermatogenesis in mosquitoes takes 9-10 days provided a basis for the release of sterile males, as it was feared that a shorter duration might produce viable sperms and make the technique unsuitable<sup>1</sup>.

By utilizing the technique of ovarian transplantations, it was demonstrated that dominant lethal mutations rather than gonotrophic hormones were involved in inducing sterility in mosquitoes<sup>2</sup>. These basic researches found application in the field experiments conducted in Delhi on the feasibility of the genetic control of mosquitoes. Although at that time mass production of *Culex quinquefasciatus* was possible, the available techniques of separating males from the male-female mixture were highly unsatisfactory and impractical for the envisaged large scale release of sterile males. To overcome this obstacle, Sharma invented a cheap, simple and ingenious device which could sex millions of mosquitoes in a short time<sup>3</sup>; during the extended trials in Delhi, the device sexed mosquitoes by the millions with an accuracy of > 99.7% males. At the same time, he developed methods for the sterilization of mosquitoes, and demonstrated that chemosterilized males were sexually competitive and biologically fit for inducing sterility in the wild

populations<sup>4</sup>. A major hurdle still remained, a field study having demonstrated that pre-inseminated females migrated to the villages in large numbers. Because of the monogamous behaviour of females, the released sterile males would not be able to sterilize the immigrants. These immigrants would produce viable progeny<sup>5</sup>. To protect villages from the immigrants, Sharma established a 3-km wide breeding free zone around the experimental and comparison villages for an extended period of about 6 months<sup>6</sup>. The study provided vital information on the biology of *Culex quinquefasciatus*, particularly in regard to its dispersal capacity and showed that the sterile male technique was suited for confined mosquito populations or mosquitoes with poor dispersal behaviour, such as *Aedes aegypti* or *Anopheles stephensi*<sup>7</sup>. During the same period, there was the apprehension that the cumulative amount of non-detectable residues of the harmful chemicals used in the sterilization of mosquitoes may become an environmental hazard, if releases by the millions were contemplated. To overcome this problem, a simple method for neutralizing the alkylating agents by dipping mosquito pupae, in acidic water, was developed<sup>8</sup>.

The establishment of a centre exclusively for research on malaria provided an opportunity to advance the



field of malaria epidemiology and control in India, which had remained neglected as a result of the near eradication of malaria in early 1960s. *Anopheles culicifacies*, the major vector of malaria, responsible for about 70% malaria cases in the country, was colonized for the first time in India<sup>9</sup>. This followed the discovery that *A. culicifacies* is the complex of at least three sibling species and that sibling species show variation in their biology, which has direct relevance to the epidemiology and control of malaria. The distribution of sibling species in the country is being mapped, and the technique of immunoradiometric assay using monoclonal antibodies against the sporozoite stage is being applied to incriminate vectors, marginal vectors and also the parasite load in the vectors with a view to understanding the epidemiology of malaria in areas with varying degrees of malaria endemicity.

Insecticide resistance in *A. culicifacies* has become a serious obstacle in the successful control of malaria. As a short-term measure, field work was so directed as to improve the impact of spraying and cut operational costs. This resulted in the demonstration that spraying of DDT in vector populations resistant to DDT reduced vector populations and malaria and showed the importance of correct dosage application and increasing coverage of houses in achieving malaria control<sup>11</sup>. More studies are planned on the impact of DDT in vector populations resistant to HCH and malathion.

Recently, it was stated that resurgence of malaria in India was the result of green revolution. The argument was made that large quantities of DDT were sprayed on high yielding varieties of crops, which

needed protection from insect pests. Agricultural fields were the breeding ground of mosquitoes, which became resistant to DDT as a result of the spraying of crops. It was shown by an in-depth study that this contention was grossly incorrect<sup>12</sup> and the reasons for the resurgence of malaria were outlined<sup>13</sup>.

Studies on malaria endemicity showed that the peak of *Plasmodium vivax* was followed by that of *P. falciparum*, and that falciparum malaria was on the increase in north India. It was also found that in many parts of the country, the incidence of malaria was extremely high and the NMEP's surveillance was recording only a fraction of the total incidence. There was a high incidence of atypical malaria cases in the community. The laboratory services were not detecting parasite positive cases and the modified plan of operations was not performing satisfactorily<sup>14</sup>.

Parasitological surveys revealed that in addition to poor examination of slides, the backlog of unexamined slides constituted a major obstacle in the treatment of malaria. As an aid to early diagnosis, a vital staining technique for malarial parasite was developed. This technique is rapid and simple and can be used in the field for on the spot diagnosis and treatment of parasite positive cases. Currently, field trials are in progress and encouraging results have been obtained. It is envisaged that the adoption of this technique would eliminate the need for presumptive treatment and reduce drug pressure, thereby delaying the onset of drug resistance<sup>15</sup>.

Nationwide resurgence of malaria has clearly shown that insecticidal spraying as a means of controlling malaria is at best a short-term measure, the main reasons

being the capacity of mosquitoes to evolve multiple resistance, and the escalating cost of insecticides. Sharma is currently engaged in developing an alternative strategy for malaria control without the use of insecticides, involving the community in vector control operations through health education. In the first year, malaria transmission was interrupted in about 25,000 population living in seven villages of Gujarat. The study has now been extended to the entire Nadiad Taluka comprising 3.5 lakh population living in 100 villages. The strategy, besides helping to control malaria and other vector-borne diseases, promotes social forestry, fish culture, inculcates scientific temper, generates employment and maintains the ecological integrity of the area at an affordable cost and has proved cost effective. Sharma proposes to test the feasibility of this technique in a few more ecological settings of the country in the coming years.

### Selected Publications

1. Sharma V P, Hollingworth R M & Paschke J D, Incorporation of tritiated thymidine in male and female mosquitoes, *Culex pipens*, with particular reference to spermatogenesis, *J Insect Physiol*, **16** (1970) 429-36.
2. Rai K S & Sharma V P, Cytogenetic effects of chemosterilants in mosquitoes. III. Development of transplanted ovaries in normal and chemosterilized females of *Aedes aegypti*, *J Genet*, **60**(3) (1971) 266-71.
3. Sharma V P, Patterson R S & Ford H R, A device for the rapid separation of male and female mosquito pupae, *Bull Wld Hlth Org*, **47** (1972) 429-32.
4. Sharma V P, Patterson R S, Grover K K & LaBrecque G C, Chemosterilization of the tropical house mosquito *Culex pipens fatigans* Wied, laboratory and field cage studies, *Bull Wld Hlth Org*, **48** (1973) 45-48.
5. Sharma V P, Insemination rate in *Culex pipens fatigans* Wied moving from wells to the villages, *J commun Dis*, **9** (1977) 128-31.
6. Sharma V P, A review of the work done on the protection of the experimental areas from infiltration of mosquitoes with particular reference to genetic control of *Culex pipens fatigans* Wied in Delhi Union Territory villages, *J commun Dis*, **6** (1974) 127-35.
7. Sharma V P, Field experiment with thiotapasterilized *Culex quinquefasciatus* in India, in *Integrated mosquito control methodologies*, edited by Marshal Laird & James W Miles (Academic Press Inc, London) 1985, 117-40.
8. Sharma V P, Elimination of aziridine residues from chemosterilized mosquitoes, *Nature, Lond*, **261**(5556) (1976) 135.
9. Ansari M A, Mani T R & Sharma V P, A preliminary note on the colonization of *A. culicifacies* Giles, *J commun Dis*, **9** (1977) 206-7.
10. Subbarao S K, Vasantha K, Adak T & Sharma V P, *Anopheles culicifacies* complex: Evidence for a new sibling species, species C, *Ann ent Soc Am*, **76**(6) (1983) 985-88.
11. Sharma V P, Uprety H C, Nanda N, Raina V K, Parida S K & Gupta V K, Impact of DDT spraying on malaria transmission in villages with resistant *Anopheles culicifacies*, *Indian J Malariol*, **19** (1982) 65-68.
12. Sharma V P & Mehrotra K N, Return of malaria, *Nature, Lond*, **298** (1982) 210.
13. Sharma V P & Mehrotra K N, Malaria resurgence in India—A critical study, *Soc Sci Med* (1985) in press.
14. Sharma V P, Choudhury D S, Ansari M A, Malhotra M S, Menon P K B, Razdan R K & Batra C P, Studies on the true incidence of malaria in Kharkhoda (Distt Sonapat, Haryana) and Kichha (Distt Nainital, UP) Primary Health Centres, *Indian J Malariol*, **20** (1983) 21-34.
15. Sharma V P, Vital staining of malaria parasite, *Indian J Malariol*, **20** (1983) 83-84.



# Har Dayal Srivastava

Srivastava began his research career in helminthology as a Lady Tata Memorial Research Scholar at the University of Allahabad (1933-35). He got his DSc from the same university in 1938. He worked as Helminthologist, Senior Professor of Parasitology, Head, Division of Parasitology and Director, Indian Veterinary Research Institute, Mukteswar/Izatnagar. During 1970-74, he was Emeritus Scientist of the Indian Council of Agricultural Research.

Srivastava has been engaged in conducting and guiding research in helminthology for over 53 years and has covered its various aspects: survey, morphology, taxonomy, reservoir hosts, carriers, life cycle, prenatal infection, pathogenicity, etiology of certain diseases of obscure origin, treatment and control, including biological control, host-parasite relationships, immunity, zoonosis, age and acquired resistance, effects of deficiencies in feeds of poultry and ruminants on their susceptibility to worm infections, etc.

Some of his more salient research achievements are mentioned below in brief.

## (1) New Diseases

(i) *Unique form of dermatitis in cattle* : It is a chronic form of skin disease affecting cattle. The skin becomes very tender and peels off in patches at the

slightest injury or abrasion, leaving bright-red bleeding patches which attract swarms of flies. The usefulness of the affected animal is reduced considerably on account of numerous wounds and constant irritation. The severity of the disease and the number of larvae in the skin show marked seasonal variations, the lesions becoming quiescent during winter months. This condition has been proved to be due to heavy infection with an unsheathed microfilaria. No such disease was known previously. It is referred to in literature as 'Srivastava's dermatitis of cattle'.

(ii) *Verminous pneumonia in Indian buffaloes* : The occurrence of lungworms in sheep, goats, cattle, equines, pigs, cats and dogs has been known for a long time in India and other countries. But the occurrence of verminous pneumonia in buffaloes and the identity of the causative parasite were recorded by Srivastava for the first time.

(iii) *Periodic ophthalmia in equines* : A few cases of periodic ophthalmia in army horses were investigated. From histopathological studies of the affected eyes, the condition was found to be due to an unsheathed microfilaria.

## (2) Elucidation of the Life Cycles of Some Helminth Parasites of Domestic Animals and Man

Srivastava worked out the life histories of some important parasites.

---

Formerly, Director, Indian Veterinary Research Institute, Mukteswar/Izatnagar; Residence : F/24, Hauz Khas Enclave, New Delhi-110016.

(i) *Stephanofilaria assamensis* : This is the causative parasite of humpsore of Indian cattle. Species of *Stephanofilaria* occur in USA, USSR, Japan, India and several countries in the Far East. They cause skin sores in cattle, buffaloes, goats, equines and elephants. Researches to discover the transmitting agents of the worms in several countries for decades had been abortive. It has been conclusively proved by Srivastava that *S. assamensis* is transmitted by a lapping fly—*Musca conducens*. In other filariids, blood-sucking arthropods serve as intermediate hosts, but in *Stephanofilaria* species, a lapping fly, following biting flies which open up wounds, transmits the infective larvae. Subsequently, Ivashkin and Khronova (1963) in USSR, Hibler (1964) in USA and Kono (1968) in Japan reported similar findings.

(ii) *Gastrodiscoides hominis* : This is the intestinal Amphistome of man and pig. Srivastava worked out the life cycle of this parasite in detail; the Planorbid snail, *Helicorbis coenosus*, was found to serve as the intermediate host under both experimental and natural conditions.

(iii) *Artyfechinostomum sufrartyfex* : This intestinal trematode of man and pig is of great zoonotic importance, all the recorded cases of human infection with it having proved fatal. The pig serves as its reservoir host. Its life history has been worked out in detail. *Indoplanorbis exustus* has been proved to serve as its first intermediate host and snails and tadpoles as the second intermediate hosts in which the metacercariae are formed.

(iv) *Schistosomes and schistosomiasis of veterinary importance* : Human schistosomiasis is not a very serious problem in India and only its urinary form

occurs in a small village, Gimbi, in Ratnagiri district. However, animal schistosomiasis is a serious problem. In India, eight species of bloodflukes are known to infect domestic animals, the largest number occurring in any one country. Extensive studies have been conducted on various aspects of the problem, including elucidation of the life cycles of *Schistosoma indicum*, *S. incognitum*, *S. nasale*, *Orientobilharzia turkestanicum*, and *O. dattai*, the last referable to a new genus and species, pathogenicity, host-parasite relationship and treatment.

The specific diagnosis of bloodfluke infection in animals by the detection of eggs in the faeces is difficult and tedious. An easy method for the specific identification of miracidium based on its morphological characteristics has been developed.

(v) *Amphistomes and amphistomiasis of domestic animals* : Several species of Amphistomes referable to different genera are known to parasitise man, sheep, goats, cattle, buffaloes, elephants, equines and pigs in India. While in adult stage they are almost innocuous, in their immature stages they are very pathogenic to ruminants and cause heavy mortality among the infected animals, particularly the young stock. The life cycle of *Cotylophoron cotylophorum*, a pathogenic Amphistome of ruminants, has been worked out, its pathogenicity studied experimentally and a biological control of it found. The life histories of several other Amphistomes, including two species each of *Palamphistomum* and *Sureshiella* and of the type species of *Duttiella*, all of ruminants, have been elucidated; the last three genera are new to science. The life history of *Pseudodiscus collinsi*, an



Amphistome of equines, has also been worked out. Different species of aquatic snails have been found to serve as their intermediate hosts.

In the life cycles of all digenetic trematodes, snails act as the first intermediate hosts. For a study of the chemotactic attraction of miracidia to their snail hosts, a special breaker, 'Srivastava's breaker', has been devised.

(vi) *Prenatal infection of calves with Neoascaris vitulorum*: This large roundworm of cattle and buffaloes is highly pathogenic and causes mortality among their calves. It has been proved experimentally that the infection with it is prenatal.

### **(3) New Parasites of Fresh-Water and Marine Food-Fishes**

An extensive study of the trematode parasites of Indian fresh-water and marine food-fishes has revealed the occurrence of a large number of parasites which are new to science. Their morphology has been described in detail and they have been referred to three new families, over 50 subfamilies and a large number of genera and species. Some of them are unique in their morphology.

### **(4) Some New/Unrecorded Parasites of Domestic Animals**

(i) *Prosthogonimus indicus*, a new fluke infecting the oviduct of fowls: This trematode produces acute inflammation in the oviduct. As a result, the eggs laid by the infected birds are soft-shelled and get damaged easily. Sometimes, one or two specimens of the parasite get enclosed in an otherwise normal egg.

(ii) *Parafilaria sahii*: This is a new parasite causing cutaneous bleeding (parafilariasis) in buffaloes.

(iii) *Onchocerca cervicalis*: Though the condition known as "Khojlee" or Lichen tropicus in equines was known to occur in India for a long time, the occurrence of the causative parasite, *O. cervicalis*, has been recorded with the recovery of the adult worm from the ligamentum nuchae of the affected horses.

(iv) *Ornithostrongylus quadriradiatus*: This is a highly pathogenic roundworm of pigeons, causing catarrhal enteritis and debilitating diarrhoea. Fancy and valuable breeds of pigeons are particularly susceptible to its infection. Its occurrence in India had not been reported previously.

### **(5) Effect of Nutrition of the Host on Its Susceptibility to Helminthic Infection**

The effects of deficiencies of protein, calcium and vitamin A, separately and in combination, in the feeds of poultry, on their susceptibility to infection with the large intestinal roundworm and caecal-worm, have been studied experimentally. Their susceptibility to infection with these worms was found to increase appreciably when they were fed on deficient feeds. A similar effect of deficiencies of trace elements, vitamin A, protein, calcium and phosphorus in feeds on the natural resistance of sheep to infection with *Fasciola gigantica* was observed.

### **(6) Age and Acquired Resistance of Animals to Certain Helminthic Infections**

(i) The age resistance of chicks to infection with *Ascaridia galli* has been studied experimentally. It has been found

that the healthy birds develop strong resistance to infection with the worm if they are kept free from it for the first three weeks of life.

(ii) The chicks under three weeks of age were found to develop acquired resistance to the infection after an initial infection.

(iii) Certain species of snails have been observed to become refractory to infection with the miracidium of some trematodes when they become adult.

Srivastava is a Fellow of the National Academy of Sciences of India, and the Indian National Science Academy. He has been President, Zoological Society of India; Helminthological Society of India; Founder President, Indian Society for Parasitology; and President, Section of Zoology, Indian Science Congress Association (1960). Among the awards won by him are the Bhalerao Memorial Gold Medal and the Rafi Ahmed Kidwai Prize for pioneering research work in Agricultural and Animal Sciences. His researches have been extensively referred to in literature. "As a tribute to Dr Srivastava's international standing as a parasitologist, a number of senior parasitologists in India and many foreign countries, who are acknowledged leaders in their fields of speciality, have contributed important research papers to the *Festschrift*" (published on his 60th birthday) [B P Pal (1968)].

### Selected Publications

1. Srivastava H D, A study of the life history and pathogenicity of *Cotylophoron cotylophorum* (Fischöeder 1901) Stiles and Goldberger (1910) of Indian ruminants and a biological control to check the infestation, *Indian J vet Sci Anim Husb*, **8** (1938) 381-85.
2. Srivastava H D, The morphology and systematic relationship of a new parasite *Waretrema piscicola*, gen et sp Nov, referable to a new Family Waretrematidae of digenetic trematodes, *Indian J vet Sci Anim Husb*, **9** (1939) 169-72.
3. Srivastava H D, Cutaneous microfilariasis in Indian cattle, *Indian J vet Sci Anim Husb*, **9** (1939) 389-91.
4. Sinha P K & Srivastava H D, Studies on *Schistosoma incognitum* Chandler, 1926, Part II. On the life history of the bloodfluke, *J Parasit*, **46** (1960) 629-41.
5. Deo P G & Srivastava H D, Studies on the acquired resistance of chickens to *Ascaridia galli* (Schrank, 1788) Freeborn, 1923, *Indian J vet Sci Anim Husb*, **31** (1961) 203-11.
6. Dutt S C & Srivastava H D, Morphology and life history of the mammalian bloodfluke *Orientobilharzia dattai*. Part I. Morphology of the adult, egg and miracidium, *Indian J vet Sci Anim Husb*, **31** (1961) 288-303.
7. Dutt S C & Srivastava H D, On the epidermal structures of the miracidia of six species of mammalian Schistosomes and a new technique of diagnosis of animal schistosomiasis, *Indian J Helminth*, **13** (1961) 61-73.
8. Srivastava H D & Dutt S C, Studies on *Schistosoma indicum*, *Indian Counc Agric Res, Res Series No 34* (1962) 1-91.
9. Deo P G & Srivastava H D, Studies on the effects of different deficient feeds on the natural resistance of *Ascaridia galli*, *Indian J vet Sci Anim Husb*, **32** (1962) 54-69.
10. Dutt S C, Srivastava H D *et al*, Chemotherapy of *Schistosoma indicum* infection in sheep, *Ann Biochem exp Med*, **23** (1963) 439-46.
11. Srivastava H D & Dutt S C, Studies on the life history of *Stephanofilaria assamensis*, the causative parasite of humpsore of Indian cattle, *J Helminth*, **33** (1963) 173-77.
12. Dutt S C & Srivastava H D, The intermediate host and cercaria of *Gastrodiscoides hominis* (Trematoda-Gastrodiscidae), *J Helminth*, **40** (1966) 45-52.
13. Deo P G, Srivastava H D *et al*, Studies of the effects of feeds deficient in trace-elements, vitamin-A, protein, calcium and phosphorus on the natural resistance of sheep to common liver-fluke, *Fasciola gigantica* Cobbold, 1885, *Indian J vet Sci Anim Husb*, **37** (1967) 351-59.
14. Srivastava H D & Malviya H C, Treatment of humpsore in cattle caused by *Stephanofilaria assamensis*, *Indian vet J*, **45** (1968) 484-88.
15. Dutt S C & Srivastava H D, The life history of *Gastrodiscoides hominis* (Lewis and Mc Connel 1876) Leiper (1913), the amphistome parasite of man and pig, *J Helminth*, **46** (1972) 35-46.



## P N Srivastava

Srivastava began his research career by working on the morphology and histology of air bladder in fishes. He showed that the oval and pneumatic ducts in the air bladder cannot be regarded as homologous structures.

He shifted to the area of radiation biology in the early sixties. His early work was devoted to the effect of radiation on various tissues at cellular and subcellular levels, which raised doubts about the radioresistance of liver. It is no longer considered as a radioresistant organ and the results have been incorporated in various textbooks.

Tritium, the heavy isotope of hydrogen, is a byproduct of nuclear technology which during fuel reprocessing is released to some extent in the environment. This has generated continued interest in knowing the effect of such isotopes on human health, especially at low dose levels. For a long time, the Relative Biological Effectiveness (RBE) of tritium compared to that of cobalt-60 or hard X-rays was considered to be 1. Theoretical studies of nuclear physicists had predicted this to be more than 1. This view was contested by many and the value was not enhanced. Srivastava's work on the ovary of mice along with that of some other workers showed and confirmed that the RBE of tritium could exceed 2, which was

accepted at the International Congress of Radiation Research held in Tokyo in 1979. Srivastava's organ culture work on the ovary has shown that the RBE could even be closer to 4 at very low doses.

Numerous efforts have been made to explain the genesis of late effects in various tissues. Srivastava followed the activity and characteristics of hexokinase isozyme through five generations of mice. The levels of enzyme activity varied widely showing that tritium at low doses induced conformational changes in the protein structure. Efforts are on to measure tritium incorporation into the enzymes and also to see if links can be established between the mutational effects of low-dose tritium irradiation and the level of regulatory enzymes in cells.

The consequences of lipid peroxidation in biomembranes for the functioning of membrane-bound enzymes (after irradiation) are of great significance. The possibility of post-irradiation damage to the protein part by hydroperoxidases left in the irradiated lipids cannot be ruled out and is being looked into currently. Lipid peroxidases could not be measured in mammalian cells after irradiation with biologically significant doses.

The clinical application of radioprotective drugs in cancer therapy is very much

limited for want of drugs having the twin properties of low toxicity and high value of dose modification factor. Srivastava has been actively engaged in determining the mechanism of action of certain radioprotective drugs. His work on MPG and WR-2721 has shown that the activity of acid and alkaline phosphatases in mouse liver and kidney homogenates is altered significantly when it is assayed after the addition of the drugs. It has been suggested that the drugs bind with the enzyme either at the substrate binding site or elsewhere in the enzyme molecule in such a way that the activity is changed.

It is well understood that radiation-induced membrane and DNA damage is a function of both dose and dose rate. However, the question whether DNA damage is more important or membrane damage still remains unresolved. Srivastava's work has shown that both MPG and WR-2721 are good radioprotectors and free radical scavengers, but apparently do not seem to be effective against DNA damage at high dose rates. Hence, DNA and membrane appear to be related inversely to each other *vis-à-vis* dose rate. Further work in this area is in progress.

### Selected Publications

1. Srivastava P N, The air bladder of Mugilidae and the relationship of the oval and pneumatic duct, *Trans Am microsc Soc*, **76** (1957) 346-54.
2. Srivastava P N, Thyroidal control of radiophosphorus metabolism in salmon, *Nature, Lond*, **185** (1960) 612-13.
3. Srivastava P N, Influence of thyroid on radiophosphorus metabolism in fish, *Nature, Lond*, **188** (1960) 512-13.
4. Srivastava P N & Tachi C. Sodium excretion as a factor for death after irradiation in fish, *Nature, Lond*, **202** (1964) 1234-35.
5. Srivastava P N & Tachi C, Effect of irradiation on the excretion of radiosodium from the gills of goldfish, *Carassium auratus* L. *Radiat Res*, **23** (1964) 222-31.
6. Srivastava P N & Ram S, Effect of Co-60 irradiation on the liver of *Heteropneustes fossilis* Bloch, *Strahlentherapie*, **137** (1969) 499-508.
7. Sugahara T & Srivastava P N, 2-Mercaptopropionylglycine (MPG) : A review on its radioprotective action against ionizing-radiation, in *Modification of radiosensitivity of biological systems* (International Atomic Energy Agency), 1976, 77-86.
8. Srivastava P N, Studies on the effect of radiophosphorus (P-32) on Swiss albino mice during postnatal development, *Presidential Address, Section of Zoology, Entomology and Fisheries, Indian Science Congress*, 1977.
9. Srivastava P N, Tritium—A possible pollutant of the future, *Presidential Address, Biological Sciences, National Academy of Sciences, India*, 1978.
10. Sharan R N & Srivastava P N, Effects of tritiated water ingestion on mice: I. Hexokinase activity changes in brain and liver, *J Radiat Res*, **21** (1980) 231-38.
11. Kapoor G & Srivastava P N, Effect of low-level tritium exposure on the ovary of mice *in vitro*, *J Radiat Res*, **21** (1980) 163-69.
12. Srivastava P N, Sharan R & Pozzi L, Effects of tritiated water ingestion on mice. II. Damage at cellular *vis-à-vis* subcellular level monitored upto four generations, in *Nuclear medicine and biology*, edited by C Rayaud (Pergamon Press, London), 1982, 2989-92.
13. Sharan R N & Srivastava P N, Effect of tritiated water ingestion on mice. III. Hexokinase isozymes in brain, liver and spleen upto five generations, *Int J Radiat Biol*, **46** (1984) 83-93.
14. Kapoor G, Sharan R N & Srivastava P N, Histopathological changes in ovary following acute and chronic low-level tritium exposure to mice *in vivo*, *Int J Radiat Biol*, **47** (1985) 197-203.
15. Ayene S I & Srivastava P N, Radioprotective effect of 2-Mercaptopropionylglycine (MPG) on the radiation-induced microsomal lipid peroxidation, *Int J Radiat Biol*, **48** (1985) 197-205.



## U S Srivastava

Srivastava has made extensive and original contributions in the areas of insect endocrinology and insect developmental morphology. He made the first authentic demonstration of the moulting glands in Coleoptera. He was also the first to demonstrate experimentally the true juvenilising effect of the juvenile hormone and juvenoids in higher Diptera. He has shown that the difference in the response of different insects to juvenoid treatment is related to the differences in the habit and habitat of the insects. He has recently demonstrated that the treatment of embryos of *Earias fabia* brings about disruption in the developmental process at certain well-defined stages during the embryonic and post-embryonic development of the insect and that the stage at which such disruption occurs is related to the stage of embryonic development at which juvenoid treatment is given and the quantity of compound administered. However, the stages affected are generally those at which rapid cell proliferation and/or differentiation and secretion take place. He has also shown that the fecundity of insects is severely inhibited by juvenoid treatment of eggs, larvae, pupae and adults. Recently, he has studied the effect of juvenoid treatment on the organogenesis of various organs, specially gut structures and compound eyes and has shown the inhibitory effect of

the treatment on the division and secretory activities of different kinds of cells during the development of these organs. In aphids, he has demonstrated that morph determination and wing development are related to JH titre and its higher titre prevents wing development and hence leads to apterae production. Earlier, he had provided the first evidence to indicate that the neurosecretory cells of the brain, on one hand, and those of the thoracic and abdominal ganglia on the other, have different roles.

His earlier work on the post-embryonic development of reproductive organs of several insects and embryonic and post-embryonic development of Malpighian tubules had been appreciated and quoted widely.

### Selected Publications

1. Srivastava U S, Prothoracio glands in *Tenebrio molitor* Linn. (Coleoptera, Tenebrionidae), *Nature, Lond*, **181** (1958) 1668.
2. Srivastava U S, The prothoracio glands of certain Colepteran larvae, *Q J micr Sci*, **100** (1959) 51-64.
3. Srivastava U S, Secretory cycle and disappearance of prothoracic gland in *Tenebrio molitor* Linn. (Coleoptera, Tenebrionidae), *Experientia, Basel*, **26** (1960) 445.
4. Srivastava U S & Bahadur J, The development of Malpighian tubules in *Dysdercus koenigii* (Hemiptera, Pyrrhocoridae), *Q J micr Sci*, **102** (1961) 347-60.

5. Srivastava U S & Om Prasad, The activity of the neurosecretory cells of *Periplaneta americana* in relation to ovarian cycle, *Proc. natn Acad Sci India*, **35** (1965) 399-410.
6. Srivastava U S & Khare M K, The development of Malpighian tubules and associated structures in *Philosamia ricini* (Lepidoptera, Saturniidae), *J Zool, Lond*, **150** (1966) 145-63.
7. Srivastava U S & Gilbert L I, Juvenile hormone: Effects on a higher Dipteran, *Science*, **161** (1968) 61-62.
8. Srivastava U S & Gilbert L I, The influence of juvenile hormone on the metamorphosis of *Sarcophaga bullata*, *J Insect Physiol*, **15** (1969) 177-89.
9. Srivastava R C & Srivastava U S, Effect of a juvenile hormone analogue on the oviposition of *Trogoderma granarium* Everts, *Proc natn Acad Sci India*, **43(B)** (I and II) (1973) 105-12.
10. Srivastava U S & Srivastava R C, Effect of a juvenile hormone analogue on the biology of *Trogoderma granarium* Everts, *Proc natn Acad Sci India*, **44** (I and II) (1974) 99-120.
11. Srivastava U S & Singh S V, The effect of juvenile hormone on the development of peritrophic membrane in *Sarcophaga ruficornis* F, *Natn Acad Sci Lett*, **1**(1) (1978) 42-43.
12. Srivastava U S, Srivastava R C, Prasad S S & Srivastava P, Endocrinological adaptations in insects, *Experientia*, **35** (1979) 1301-2.
13. Srivastava U S & Singh S V, The effect of juvenile hormone on metamorphic changes in the hindgut of *Sarcophaga ruficornis* (Sarcophagidae: Diptera), *Natn Acad Sci Lett*, **2**(5) (1979) 197-98.
14. Srivastava U S & Chhibber Neelam, Effect of a juvenoid on wing development and differentiation in an aphid *Aphis nerii*, *Indian J exp Biol*, **18** (1980) 814-17.
15. Srivastava U S & Singh S V, Effect of juvenile hormone on the metamorphic changes in the midgut of *Parasarcophaga ruficornis* (Diptera, Sarcophagidae), *Acta ent Bohemoslov*, **77** (1980) 222-27.
16. Srivastava U S & Tribhuvan Singh, Effect of a juvenoid on post-natal development of *Aphis craccivora* Koch (Homoptera: Aphididae), *Indian Zoologist*, **5** (1 and 2) (1981) 141-50.
17. Srivastava U S & Srivastava Punam, Effect of administration of a juvenoid to the last instar larva of *Earias fabia* Stoll, *Proc Indian natn Sci Acad*, **47(B)**(6) (1981) 799-810.
18. Srivastava U S & Prasad S S, Effect of a juvenoid on the development of compound eyes in *Spodoptera litura* Fabr., *Proc Indian natn Sci Acad*, **91**(4) (1982) 334-48.
19. Srivastava U S & Shukla Kusum M, Effect of a juvenoid on the embryogenesis of *Earias fabia* F, *J Insect Physiol*, **28**(4) (1982) 299-304.
20. Srivastava U S & Prasad S S, Delayed effects of juvenoid treatment of larval instars of *Spodoptera litura* (Fabr.) on development and reproduction, *Proc natn Acad Sci, India*, **54(B)** (1984) 272-87.





**Sectional Committee—VIII : Medical Sciences**  
**(*Basic and Clinical Medical Sciences :***  
***Anthropology, Psychology*)**





## S C Agarwal

The mechanism of circulatory failure in diphtheria intoxication has remained a controversial problem since the discovery of diphtheria toxin. It was felt by earlier workers that damage to blood vessels is the main likely cause of circulatory collapse. Agarwal and Holt<sup>1</sup> suggested the possibility that the circulatory failure may be due to diminution of the reactivity of smooth muscle cells in the arterioles and capillaries to the naturally liberated vasoconstrictor amines, adrenaline and nor-adrenaline. They found that in positive Shick reactions obtained by injecting diphtheria toxin intradermally into guineapigs, the skin vessels became refractory to adrenaline and nor-adrenaline. They thought that the toxin may be acting on the smooth muscle cells of the arterioles and capillaries. Agarwal and Nasmyth<sup>2</sup> further showed that the diphtheria toxin reduced the responses of cat's nictitating membrane to adrenaline or nor-adrenaline either liberated naturally at sympathetic nerve ends on preganglionic stimulation or introduced from outside. A similar effect was produced in the rabbit uterus. It is unlikely that these effects are produced by reduction in the qualities of sympathomimetic amines released at the nerve ends, because the responses to injection of adrenaline as well as those to nerve stimulation are reduced. The increased destruction of sympathomimetic

amines is also unlikely to be the explanation, as the effects of acetylcholine, KCl and BaCl<sub>2</sub> are also diminished. The inhibition in the response to sympathomimetic amines is specific, because the effect of toxin on the cat's nictitating membrane responses could be blocked by active and passive immunization with diphtheria antitoxin or by the injection of toxin-antitoxin mixtures. It was thought that the reduction in the contractility in smooth muscle could be affected by a defect in the movement of potassium ions across the cell membrane. The finding in the diphtheria intoxicated guineapigs that the potassium content of the thoracic and abdominal aortic wall is considerably higher than in any normal animal, while in other tissues, including the uterus and the arteries, it is not affected, suggests that the vascular system is much more sensitive to the action of toxin. On the basis of these observations, a new theory was propounded for the toxæmic shock in diphtheria. It was suggested that the smooth muscle cells of the arterioles and capillaries became refractory to the action of adrenaline and nor-adrenaline. There is also a defect in the permeability of cell membrane to potassium ions in some tissues and inhibition of the loss of potassium from smooth muscle cells, resulting in an increase in the potassium content of the cells and their decreased

---

Director, Professor & Chairman, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar-190011; Residence : 3, Harmony, Rajbagh, Srinagar.



contractility. This results in vasodilation of arterioles and capillaries and the consequent toxæmic shock.

### **Mechanism and Pathophysiology of Diphtheritic Paralysis**

Agarwal and Pryce<sup>3</sup> showed that intracerebral injection of diphtheria toxin in rats produces a clinical syndrome of ascending paralysis. The nerve cells in the brain and spinal cord show gross degenerative changes. It seemed likely that even before any histological damage is evident, the toxin may interfere with the physiological processes in the nerve cell. These alterations in the normal physiology of the neurone might be responsible for defective conduction of nerve impulse and diphtheritic paralysis. Since the release and destruction of acetylcholine are the main mechanisms involved in the initiation and conduction of nerve impulses, it was thought worthwhile to test the effects of toxin on cholinesterase activity. The results show that toxin does not interfere with the activity of these enzymes. It was, therefore, possible that the cause of diphtheritic paralysis is a defect in the release or synthesis of acetylcholine. In 1964, Agarwal<sup>5</sup> found that diphtheria toxin inhibited the *in vitro* synthesis of acetylcholine in rat brain slices. It does not affect the conversion of bound into free acetylcholine. The cause of inhibition of acetylcholine synthesis may be (i) interference with the acetylation of CoA into acetyl CoA, (ii) interference with the activity of choline acetylase, and (iii) diminution of any of the parent substrates, such as choline or acetate. It was found that addition of diphtheria toxin did not lower the yield of acetylcholine in a choline acetylating system. This would mean that the toxin does not inhibit the mechanism

of acetylcholine synthesis directly in acetylation of CoA or the release of energy from ATP and the enzymic activity of choline-acetylase. It is suggested that diphtheritic paralysis may be due to diminished synthesis of acetylcholine in nerve cells due to a deficiency in the parent substrates, i.e. acetyl CoA and choline, which are necessary for acetylcholine synthesis. Later, this was substantiated by the fact that there is an increase in  $\alpha$ -keto acids in guinea pig tissues, such as liver, heart, spleen, kidney and adrenals. It is fairly marked in spleen and adrenals<sup>6</sup>. On the basis of these observations, it was suggested that diphtheria toxin causes a block in the conversion of pyruvate into acetyl CoA, resulting in increased accumulation and non-utilization of pyruvate and diminished synthesis of acetyl-CoA, and thereby of acetylcholine.

### **Development of L-phase Lysate Vaccines of *V. cholerae* and Ribonucleic Acid Vaccines**

The conventional cholera vaccine is a whole cell vaccine. It contains equal number of *V. cholerae* subtype Ogawa and Inaba organisms. A newer concept in vaccine therapy was introduced by suggesting the use of lysates of L-forms of bacteria. The L-forms are cell wall deficient mutants of bacteria. Their use in immunization against cholera was investigated. Agarwal and Ganguly<sup>7</sup> immunized rabbits with disrupted L-forms of *V. cholerae* and tested their sera for the development of homologous and heterologous antibodies to *V. cholerae* and *El tor* strains. The results obtained showed that L-forms are antigenic and they produce both homologous and heterologous antibody response. The

immune responses were measured by finding the bactericidal activity of *V. cholerae*. Later, L-form lysate vaccine containing Ogawa, Inaba and Ogawa + Inaba subtypes of *V. cholerae* were tested experimentally in rabbits for the production of humoral immunity after parenteral immunization. The humoral immune responses were measured by (1) agglutinating antibodies, (2) vibriocidal antibodies, (3) anti-toxin levels, and (4) mouse potency. *V. cholerae* lysate vaccine showed good agglutinating and vibriocidal responses. The mouse potency test for Ogawa lysate vaccine had a relative potency 7.7 times more for Ogawa part and 1.4 times more for the Inaba part compared to the standard reference cholera vaccine. The anti-toxin levels were variable. Thus, it appeared that the L-form lysate vaccines are more potent antigenically than the conventional cholera whole cell vaccines. Agarwal and Ganguly<sup>8</sup> showed that the oral administration of L-form lysates experimentally also produced good humoral antibody responses. An early and high coproantibody response developed. It persisted even six weeks after immunization. There was also a considerable rise in serum haemagglutinating antibodies and these persisted in high titres up to 6 weeks. Agarwal and Ganguly<sup>9</sup> followed up this experimental work and orally immunized human volunteers with L-form lysates of *V. cholerae*. Oral administration of heat inactivated lysates of L-form Ogawa lysate cholera vaccine produced high coproantibody, serum indirect haemagglutinating antibody and vibriocidal antibodies in six human volunteers. Agarwal and Sunderaraj<sup>10</sup> reported the development of cell-mediated immunity to *V. cholerae* with ribonucleic acid-protein

fractions of *V. cholerae* L-form lysates on parenteral immunization. These authors<sup>11</sup> also reported its development even after oral immunization with ribonucleic acid-protein fractions of *V. cholerae* L-form lysates. Oral administration of single dose of ribonucleic acid-protein fractions of lysates of *V. cholerae* subtype Ogawa L-forms induced increase in cell mediated immunity in rabbits. This was evident from an increase in leukocyte migration inhibition in peripheral blood leukocytes, in macrophage migration inhibition and in microbicidal activity against *Listeria monocytogenes* in peritoneal macrophages obtained from oral immunized rabbits. Increased cell mediated immunity was induced mainly with *V. cholerae* Ogawa and ribonucleic acid protein fractions. It appeared in 20 days and persisted for at least 90 days. Agarwal<sup>12</sup> also showed that after oral immunization with ribonucleic acid-protein fractions, there was an increase in intestinal immunity in experimental animals.

These results showed that L-form lysate vaccines can be used to give protection against cholera. They produce humoral, cellular and intestinal immunity even on oral administration.

### Selected Publications

1. Agarwal S C & Holt L B, Local factors in the pathogenesis of circulatory failure in diphtheria, *J Path Bact*, **77** (2) (1959) 381-88.
2. Agarwal S C & Nasmyth P A, Some peripheral actions of diphtheria toxin, *J Physiol*, **147** (3) (1959) 511-20.
3. Agarwal S C & Pryce D M, Experimental diphtheritic paralysis in rats, *J Path Bact*, **78** (1) (1959) 171-77.
4. Agarwal S C, Effects of diphtheria toxin on acetylcholine synthesis, *J Path Bact*, **79** (2) (1960) 313-18.



5. Agarwal S C, Effect of diphtheria toxin on the utilization of single carbon sources by *Klebsiella aerogenes*, *J Path Bact*, **87** (1) (1964) 186-90.
6. Agarwal S C, Accumulation of keto-acids in tissues during diphtheria intoxication, *Nature, Lond*, **208** (5013) (1965) 914-15.
7. Agarwal S C & Ganguly N K, Agglutinin and vibriocidal responses of L-forms of *Vibrio cholerae* and *El Tor*, *Indian J med Res*, **59** (1971) 866-72.
8. Agarwal S C & Ganguly N K, Experimental oral immunization with L-forms of *Vibrio cholerae*, *Infect Immun*, **5** (1972) 31-34.
9. Agarwal S C & Ganguly N K, Oral immunization with L-forms of *Vibrio cholerae* in human volunteers, *Infect Immun*, **6** (1972) 17-20.
10. Agarwal S C & Sundararaj T, Cell-mediated immunity to *Vibrio cholerae* with ribonucleic acid-protein fractions of *V cholerae* L-form lysates, *Infect Immun*, **14** (1976) 363-67.
11. Agarwal S C & Sundararaj T, Cell-mediated immunity after oral immunization with ribonucleic acid-protein fractions of *V cholerae* L-form lysates, *Infect Immun*, **16** (1977) 527-30.
12. Agarwal S C, Immunogenicity of L-form Ogawa lysate vaccine and its ribonucleic acid protein fractions after oral immunization, *Proceedings, Conference on Experimental Cholera Vaccine*, ICDDR, Bangladesh, 1981, 57-68.

## B K Anand

Since 1951, the work carried out by Anand has been directed mostly towards extensive serial studies conducted with the ultimate aim of working out the higher nervous regulation of the various visceral activities, in general. These studies have been conducted mostly on the higher central nervous mechanisms included in the hypothalamus and the limbic system of the brain, as these regions are mostly responsible for maintaining the constancy of *milieu interieur* through regulation of the autonomic outflows, the endocrinal activities, as well as the affective behaviour.

Although this has been the general aim of the studies conducted by Anand, his work has been more specifically concentrated on working out the detailed pattern of the nervous regulation of food intake, and the various mechanisms involved in it.

As a result of these studies, Anand has published over 200 scientific papers, and a number of review articles in leading journals. He has contributed chapters to several monographs and textbooks.

### (I) Higher Nervous Regulation of Food Intake

Some of the original contributions made in this field are mentioned below.

(i) It was demonstrated that in the hypothalamus there is a dual mechanism for regulation of food intake. In the lateral hypothalamus is located an area which provides the organism the hunger mechanism or the urge to eat and this was given the name *Feeding Centre*. In the medial hypothalamus is an area which inhibits further eating. This region is activated after feeding and provides the satiety mechanism. This was, therefore, termed the *Satiety Centre*.

(ii) Feeding is the result of reflexes operating from the lower levels, which are facilitated by the feeding centre and inhibited by the satiety centre. The satiety centre also inhibits the activity of the feeding centre.

(iii) Although the basic hunger and satiety mechanisms are located in the hypothalamus, the limbic system also plays an important role in bringing about a discriminative regulation of eating. Thus, the mechanism of discriminative "appetite" is located in the limbic system.

(iv) In the hypothalamus is also located the regulating mechanism for water intake. Although this 'drinking area' is anatomically situated in the same region as the feeding area, the two mechanisms act quite independently.



(v) Thus, the regulation of alimentary behaviour (food intake) from the central nervous system is similar to the regulation of various other visceral activities which achieve maintenance of the constancy of *milieu interieur*; and the changes introduced in the internal environment by this alimentary behaviour are the ones which in turn adjust its regulation. In a starving animal, the feeding centre is active, while the satiety centre is non-active, and after taking a meal the reverse holds good. This is brought out by the nervous regulating and integrating centres being supplied with information which forms the basis for such regulation. This information is supplied to the satiety centre mostly in two ways. Firstly, the change or changes produced in the internal environment, as a result of feeding, affect the nervous regulating mechanisms. As considerable delay is involved before precise information is supplied through this channel, a second mechanism comes into play via the afferent nerves coming from the alimentary system, which transmit rapidly acting messages.

This has been demonstrated by the following observations :

(a) Distension of the stomach brings about the state of satiety. When the stomach is distended with balloons, the activity of the satiety centre increases, as observed by electroencephalographic and single neurone unit recordings. This simultaneously inhibits the electrical activity of the feeding centre. This information is routed through the gastric branches of the vagus.

The presence of gastric hunger contractions in an empty stomach does not change the activity of the

hypothalamic centre. On the other hand, an empty stomach will only show hunger contractions if the satiety centre is not activated by increased glucose utilization.

(b) The electrical activity of the satiety centre, as observed by electroencephalographic and single neurone recordings, is also increased when the level of glucose utilization in the body increases. There is a certain degree of correlation between the level of glucose utilization and the activity of the satiety centre. Reciprocal changes are produced in the activity of the feeding centre. Other hypothalamic or central nervous regions do not show any such effects in response to changes in glucose utilization.

(c) The metabolism of these hypothalamic centres has also been studied in Warburg manometers, in the fed and starving animals. It was observed that in the fed animals, the satiety centre picks up more oxygen and more glucose than the feeding centre, while the reverse is the case in the starving animals.

(d) Changes in the amino acid and fat contents of the circulating blood do not produce any change in the activity of the hypothalamic centres. Similarly, small localized changes in temperature produced in the hypothalamus do not influence the activity of these centres.

(e) The effects of some of the pharmacological preparations, influencing appetite and hunger, on the activity of hypothalamic centres, have also been studied. It has been observed that most of the preparations which influence appetite do so through changes in the level of glucose utilization.

## **(II) Higher Nervous Regulation of Autonomic and Visceral Activities**

Starting with the hypothalamic regions and gradually going over the various limbic lobe structures, the effects of stimulations and ablations of various regions have been mapped out. It has been demonstrated that both the hypothalamus and the limbic system influence the activities of all those viscera which are under autonomic regulations. Also, while in the hypothalamic region there is a distinct localization in terms of sympathetic and parasympathetic controls, the responses of visceral systems from the limbic system are quite diffuse and do not show any clearcut localization. It has, therefore, been suggested that hypothalamus is the motor area of this system, while the rest of the limbic system has integrative functions, like the functions of the neocortical association areas.

Some of the original contrioutions in this field are listed below.

### **(1) Cardio-vascular system**

(i) Frontal lobe limbic structures have a pressor effect, while temporal lobe limbic structures produce a depressor response.

(ii) Reserpine (Serpasil) produces a dual effect on the hypothalamus. It inhibits the 'sympathetic' regions and produces active facilitation of 'parasympathetic' regions.

(iii) After use of a general anaesthetic, the vascular responses become much varied. It is, therefore, not advisable to use anaesthetized preparations, but carry out stimulations in unanaesthetized animals.

### **(2) Respiratory system**

(iv) Frontal lobe structures are facilitatory, while temporal lobe structures are inhibitory.

### **(3) Gastro-intestinal system**

(v) Lesions in the pre-optic region of the hypothalamus produce acute haemorrhagic ulcers in the gastric mucous membrane.

(vi) Such ulcers (not so extensive) are also produced by lesions of the orbitomesial cortex and the amygdaloid nuclei.

### **(4) Liver functions**

(vii) Lesions in the medial hypothalamus prevent acute necrosis of the liver, which is produced after heavy malarial infections, haemolysing nearly 90% RBCs.

(viii) Lesions and stimulations of the limbic system change the liver function tests as well as liver blood flow.

### **(5) Connections of the limbic system**

(ix) It has been shown by evoked potential studies that the paleocerebellum (older part of cerebellum) projects heavily into the limbic system (older part of cerebrum).

## **(III) Nervous Regulation of Endocrinal Activities**

Some studies have also been conducted to work out the regulation of anterior pituitary secretions from the hypothalamic levels. The following are the important contributions in this field:

(i) Stimulation of median eminence of hypothalamus increases the TSH secretion, while stimulation of preoptic area and posterior maxillary region leads to inhibition of its secretion. Hypothalamus thus exercises a dual control over the secretory activity of TSH.

(ii) Study of ACTH responses after hypothalamic stimulation has also



demonstrated a similar dual hypothalamic control over its secretion.

(iii) The ventromedial hypothalamic region influences the secretions of gonadotrophins.

Feedback of gonadal hormones, on the other hand, occurs into the anterior and lateral hypothalamic regions as demonstrated by electrophysiological studies.

#### **(IV) Nervous Regulations of Affective Behaviour**

A large number of studies have been carried out to study the changes in affective behaviour after ablations and stimulations of various structures of the limbic system. All these change the affective behaviour of the animals. The changes observed have mostly been of types which have been reported by other workers also. But even in this field there have been some original contributions:

(i) The hypermotile response obtained after frontal lobe lesions is due to the involvement of head of the caudate nucleus.

(ii) The hypersexual response can be obtained not only after lesions involving the temporal lobes but also occasionally after lesions involving the frontal lobes. This response is not abolished by castration and hence is a behaviour change.

The different component parts of the limbic system, therefore, possibly act as one functional integrating unit and not as separate entities.

#### **(V) Some Observations in Yogis**

In this field again, Anand's primary interest has been to find out whether by

Yogic practices it is possible to so condition the activities of the limbic system that a voluntary control over some of these activities can be developed. Some relevant and important observations in this field have at least suggested that such a voluntary conditioning may be brought about by Yogic practices.

(i) In one Yogi, who meditated in an airtight box for 10 hours, it was observed that the O<sub>2</sub> intake and the CO<sub>2</sub> output fell to nearly 45% below the basal requirements. This suggests decrease in metabolic activities, possibly through the autonomic and endocrinal systems.

(ii) Although Yogis claiming to voluntarily stop the beating of their hearts could not be shown to do so, in one Yogi it was observed that a condition of complete heart block (as observed by EKG) could be brought about voluntarily.

(iii) In Yogis going into meditation, it was observed electroencephalographically that although the afferent stimuli are blocked and are not allowed to project to the cerebral cortex, the cortex remains in alpha activity and does not pass into the delta activity typical of sleep.

Anand has been recipient of Col Amir Chand Award (Junior) (1955); Col Amir Chand Award (Senior) (1962); G J Watumull Memorial Award (1961); Sir Shanti Swarup Bhatnagar Award (1964); Medical Council of India Silver Jubilee Research Award (1969); and B C Roy Award for Eminent Medical Man (1984). He was awarded Padma Shri in 1969.

#### **Selected Publications**

1. Anand B K & Brobeck John R, Localisation of a "Feeding Center" in the hypothalamus of the rat, *Proc Soc exp Biol Med*, **77** (1951) 323-24.

2. Anand B K & Brobeck John R, Hypothalamic control of food intake in rats and cats, *Yale J Biol Med*, **24**(2) (1951) 123-40.
3. Delgado J M R & Anand B K, Increase of food intake induced by electrical stimulation of the lateral hypothalamus, *Am J Physiol*, **172**(1) (1933) 162-68.
4. Anand B K, Dua S & Shoenberg Kate, Hypothalamic control of food intake in cats and monkeys, *J Physiol*, **127**(1) (1955) 143-52.
5. Anand B K, Higher nervous control over food intake, *Twenty-first International Congress of Physiological Sciences, Buenos Aires, Abstract of Symposia and Special Lectures*, August 1959, 196-202.
6. Anand B K, Nervous regulation of food intake, *Physiol Rev*, **41**(4) (1961) 677-708.
7. Anand B K, Influence of the internal environment on the nervous regulation of alimentary behaviour, *Second Conference on Brain and Behaviour—The Internal Environment and Alimentary Behaviour*, 1962; *Brain and Behaviour*, Vol 2, edited by MAB Brazier (American Institute of Biological Sciences. Washington DC), 1963, 43-116.
8. Sharma K M, Anand B K, Dua S & Baldev Singh, Role of stomach in the regulation of activities of the hypothalamic feeding centres, *Am J Physiol*, **201**(4) (1961) 593-98.
9. Anand B K & Pillai R V, Activity of single neurones in the hypothalamic feeding centres: Effect of gastric distension, *J Physiol, Lond*, **192**(1) (1967) 63-77.
10. Anand B K, Chhina G S, Sharma K N, Dua S & Baldev Singh, Activity of single neurones in the hypothalamic feeding centres: Effect of glucose, *Am J Physiol*, **207**(5) (1964) 1146-54.
11. Anand B K, Influence of metabolic changes on the nervous regulation of food intake, *Proc International Congress of Physiological Sciences, Leiden, Abstracts of Lectures and Symposia*, Vol 1, September 1962, 680-85.
12. Anand B K, Central chemosensitive mechanisms related to feeding, *Handbook of physiology, Sect 6, Alimentary Canal*, Vol 1 (American Physiological Society) 1963, 249-63.
13. Anand B K, Dua S & Malhotra C L, Effects of reserpine (Serpasil) on blood pressure responses evoked from hypothalamus, *Br J Pharmacol Chemother*, **12**(1) (1957) 8-11.
14. Sen R N & Anand B K, Effects of electrical stimulation of the hypothalamus on gastric secretory activity and ulceration, *Indian J med Res*, **45**(4) (1957) 507-13.
15. Anand B K, Chhina G S & Baldev Singh, Studies on Shri Ramanand Yogi during his stay in an airtight box, *Indian J med Res*, **49**(1) (1961) 82-89.
16. Wenger M A, Bagchi B K & Anand B K, Experiments in India on voluntary control of the heart and pulse, *Circulation*, **24**(6) (1961) 1319-25.
17. Anand B K, Chhina G S & Baldev Singh, Some aspects of electroencephalographic studies in Yogis, *Electroencephalogr clin Neurophysiol*, **13**(3) (1961) 452-56.



## Sachchidananda Banerjee

After the antiscorbutic vitamin, vitamin C, was synthesized and produced in large quantities, it became possible to study its metabolic role in animals and human subjects susceptible to vitamin C deficiency. A profile of several aspects of the problem studied by Banerjee is given below.

### Vitamin C-Nutrition and Utilization of Glucose

Several reports appeared indicating the usefulness of vitamin C (ascorbic acid) in the treatment of diabetes mellitus where the utilization of glucose is defective. The mechanism of action of ascorbic acid, however, was not clear. Therefore, studies were undertaken in guineapigs and monkeys, the two laboratory animals susceptible to vitamin C deficiency. When guineapigs developed scurvy after they were fed a scorbutogenic diet<sup>1</sup>, they did not utilize glucose like normally fed animals<sup>2</sup>. The utilization of glucose improved in the scorbutic guineapigs which received a small daily dose of insulin during the progress of scurvy<sup>3</sup>. Glucose utilization became normal when the scorbutic animals recovered after supplementation with ascorbic acid<sup>4</sup>. The intestinal absorption of monosaccharides was not defective in the scorbutic animals<sup>5</sup>. Scorbutic monkeys also could not utilize the fed glucose efficiently<sup>6</sup>.

When monkeys developed scurvy, the intravenously administered glucose was also not utilized efficiently. Glucose utilization became normal when the animals were cured of scurvy<sup>7</sup>.

Defective utilization of glucose in scurvy has been found to be due to defect in its intermediary metabolism. The first stage in the catabolism of glucose is the formation of glucose-6-phosphate catalyzed by glucokinase and influenced by insulin. Glucokinase activity diminished in the liver and skeletal muscle of scorbutic guineapigs<sup>8</sup>. The activity returned to normal in the animals which were given injection of a small daily dose of insulin during the scorbutic regime<sup>9</sup>. The turnover of phosphorylated intermediates of glucose metabolism in the liver in unit time was reduced below normal in the scorbutic condition with an increase in the inorganic P turnover<sup>10</sup>. This was also observed in insulin insufficiency. Fructokinase necessary for the formation of fructose-6-phosphate was not altered in the liver of scorbutic guineapigs<sup>10</sup>, although the utilization of fructose was hampered<sup>11</sup>.

At present, there is increasing evidence to indicate the involvement of insulin in the intermediary metabolism of carbohydrate at the level of the Krebs cycle. In scorbutic guineapigs, tissue contents of citric, malic and lactic acids<sup>12</sup> and urinary excretion of

---

Formerly, Professor & Head, Department of Physiology & Biochemistry and Principal, Sardar Patel Medical College, Bikaner, Rajasthan; Residence : 23B, Tarasankar Sarani, Calcutta-700037.

these acids increased considerably<sup>13</sup>, indicating involvement of the Krebs cycle. These values came to normal levels when the animals were treated with insulin during the progress of scurvy<sup>14</sup>. Increased tissue contents and urinary excretion of the keto-acids seemed to be due to decrease in the activity of citric, malic and lactic dehydrogenases in ascorbate deficiency<sup>15</sup>. Urinary excretion of citric acid came back to normal when the animals recovered from scurvy<sup>4</sup>. In scurvy, the metabolic lesion in the Krebs cycle seems to be at the level between citric acid and  $\alpha$ -ketoglutaric acid<sup>14,15</sup>, possibly due to defect in the decarboxylation of oxalosuccinic acid to  $\alpha$ -ketoglutaric acid under the action of oxalosuccinic decarboxylase<sup>13</sup>. These defects might be due to ascorbate deficiency *per se* or due to the associated diminished insulin formation.

### Vitamin C-Nutrition and Glycogen Contents of Liver and Muscle

In scorbutic guineapigs, liver glycogen was very low in comparison to that in paired-fed normal animals<sup>16</sup>. Muscle glycogen was also low. These values improved when the animals received insulin treatment or recovered after supplementation with ascorbic acid<sup>17</sup>. Deposition of glycogen in the liver of scorbutic guineapigs 4 h after feeding glucose, fructose or galactose did not improve, indicating inability to utilize the sugars for glycogen formation. The diminished tissue glycogen might be due to impaired synthesis or increased breakdown of glycogen. The rate limiting steps of glycogen synthesis and breakdown are catalyzed by uridine diphosphate glucose glycogen transglycosylase (glycogen synthetase) and

glycogen phosphorylase. The hydrolytic splitting of the 1,6-linkages of glycogen exposed by activated phosphorylase requires the action of specific debranching enzyme, amylo- $\alpha$ -1,6-glucosidase, also called  $\alpha$ -glucosidase. The combined action of phosphorylase and the debranching enzyme converts glycogen to glucose-1-phosphate. The liver glycogen synthetase activity of scorbutic guineapigs did not change from that of normal guineapigs. A significant increase in the liver phosphorylase and  $\alpha$ -glucosidase activities was found in scorbutic guineapigs<sup>17</sup>. Both in insulin-treated scorbutic animals and in animals which recovered from scurvy, the increased enzyme activities diminished. Diminished liver glycogen in scorbutic guineapigs was the result of enhanced glycogenolysis, possibly due to increased adrenaline secretion, and diminution of monoamine oxidase<sup>18</sup> in scorbutic condition. Diminished glycogen synthesis even in the presence of normal glycogen synthetase activity in ascorbate deficiency was due to diminished supply of phosphorylated intermediates necessary for the purpose<sup>10</sup>.

### Vitamin C-Nutrition and Cholesterol

In conditions of deficient utilization of glucose, fat is increasingly catabolised for supplying energy and there is abnormal lipid metabolism. Cholesterol contents of different tissues of scorbutic guineapigs differed from those of normal guineapigs<sup>19</sup>. Total body cholesterol increased significantly in the vitamin C-deficient animals without any alteration in the coenzyme A activity<sup>20</sup> and increased further when the animals were fed acetate<sup>21</sup> or butyrate<sup>22</sup>. Evidently, when the acetate pool of the body of the scorbutic guineapigs was increased, the



same was primarily disposed of by its conversion to cholesterol. Prolonged treatment with insulin during the scorbutic regime lowered the body cholesterol to normal<sup>12</sup>. When the animals recovered from scurvy after supplementation with ascorbic acid, body cholesterol came down to normal levels<sup>4</sup>.

### Vitamin C-Nutrition and Plasma Lipids

Plasma  $\beta$ -lipoproteins,  $\beta$ -lipoprotein cholesterol and plasma free fatty acids (FFA) increased considerably in scorbutic guineapigs. These changes could be reversed to normal after insulin or ascorbic acid treatments, possibly due to corrected carbohydrate utilization<sup>23</sup>. Plasma triglycerides decreased in the scorbutic condition. Similar changes in the plasma lipids were noted in scorbutic monkeys<sup>7</sup>. The changes indicated an increase in the overall rate of fat catabolism relative to the utilization of other substrates. The changes in plasma triglycerides tend to support the suggestion of diminished insulin in the scorbutic condition.

### Vitamin C-Nutrition and Insulin

The changes in the carbohydrate and lipid metabolisms in the scorbutic animal and their reversal to normal after treatment of the scorbutic animal with insulin during the progress of scurvy indicated diminished secretion of insulin in ascorbate deficiency. Insulin was extracted from the pancreas of scorbutic and normal guineapigs. In the scorbutic condition, the insulin content of pancreas diminished greatly<sup>2,16</sup>. Plasma insulin like substances greatly diminished in scorbutic monkeys and reverted to normal values when they recovered from scurvy after supplementation with ascorbic acid<sup>7</sup>. Histological

studies on the pancreas revealed increase in the number of  $\alpha$ -cells and degranulations of the  $\beta$ -cells of the islets of Langerhans which were also increased in size and number<sup>24</sup>. Insulin supplementation during the scorbutic regime prevented the changes in the islets. When the scorbutic guineapigs were allowed to recover after supplementation with ascorbic acid, the changes in the islets were reversed to normal<sup>25</sup>. This indicated that ascorbic acid is needed for normal secretion of insulin by the  $\beta$ -cells of the islets of Langerhans and the diminution of such secretion is the cause of various metabolic defects in scorbutic animals.

Vitamin C (ascorbic acid) is a sugar derivative, while insulin is a protein hormone. It is, therefore, difficult to postulate about the involvement of vitamin C in insulin secretion directly. Dehydroascorbic acid, which is normally absent in the tissues of a normal animal, appears at a considerably high level in the scorbutic animal with a simultaneous diminution in tissue glutathione, GSH<sup>26</sup>. In the plant oxidase system, GSH protects ascorbic acid and no dehydroascorbic acid is formed until all the GSH is converted to reduced glutathione GSSH. Certain enzymes in the body depend on the —SH group for their activity and if the —SH groups are inactivated, the enzymes become inactive. The activity can be restored in most cases by the addition of glutathione. Glutathione is supposed to protect the —SH group of enzymes. In toxic conditions of the body, there is increase in the dehydroascorbic acid and diminution of both glutathione and ascorbic acid of blood<sup>27</sup>. Insulin contains a large amount of cysteine and it is probable that under the influence of enzymes, cysteine is taken by  $\beta$ -cells to form the

insulin molecule. In the body, cysteine may be derived from methionine and glutathione. If the glutathione content of the body is diminished, it may interfere with the normal synthesis of insulin. Glutathione combines with dehydroascorbic acid. Dehydroascorbic acid present in the tissues of the scorbutic animal might combine with glutathione of the tissues and thereby cause a fall in the available glutathione concentration in the  $\beta$ -cells of the pancreas. The protective action of glutathione on the sulphydryl enzymes in the  $\beta$ -cells is thus further jeopardized, which might result in the moribund condition of the  $\beta$ -cells and diminished secretion of insulin in the scorbutic animal.

### **Vitamin C-Nutrition and Iron Metabolism**

When scurvy was produced in monkeys and guineapigs, all the animals developed normocytic and normochromic anemia. The peripheral blood, however, showed the presence of macrocytes, microcytes and normocytes. There was reticulocytosis<sup>28</sup>. Macrocytosis is usually due to deficiency of cyanocobalamine and pteroylglutamic acid in the diet. The diet consumed by the animals contained these vitamins. In the scorbutic guineapigs, the plasma levels of cyanocobalamine and pteroylglutamic acid did not change. Urinary excretions of these vitamins were also similar in the normal and scorbutic animals. Formiminoglutamic acid was not excreted in the urine of scorbutic guineapigs, when they were fed histidine, indicating that the animals did not suffer from pteroylglutamic acid deficiency<sup>29</sup>. Macrocytosis, therefore, was the specific effect of ascorbic acid deficiency. Microcytosis is usually associated with intestinal hemorrhage or iron deficiency.

Intestinal hemorrhage was not noticed in the scorbutic animals. The diet of the animals contained 11 mg iron per 100 g. The intake and fecal excretion of iron of the scorbutic guineapigs just balanced, indicating absence of iron deficiency. There was excessive deposition of hemosiderin in the different tissues, including intestinal villi of the scorbutic guineapigs, indicating normal iron absorption. Plasma iron, the iron binding capacity of plasma, ferritin content of liver and sideroblast percentage were low in the scorbutic animal<sup>30</sup>. All the above changes were reversed to normal when the animals were allowed to recover from scurvy. Therefore, it seems that ascorbic acid plays a role in the formation of ferritin and transference of iron from intestinal villi to plasma and its subsequent incorporation in the sideroblast of bone marrow for hemoglobin formation.

When extra iron was administered to guineapigs during the development of scurvy, the anemia became more severe, as evidenced by further diminution in hemoglobin, red cell count and packed cell volume. The aggravation of anemia was not due to intestinal hemorrhage. Treatment with iron did not increase ferritin level in liver, enhanced the deposition of hemosiderin and increased the total iron content of tissues<sup>31</sup>. Treatment of anemia with iron in the absence of the requisite quantity of ascorbic acid is, therefore, harmful. African Bantu subjects with scurvy suffer from anemia and have excessive iron deposition in tissues. Ascorbic acid is necessary for the utilization of iron for hemoglobin synthesis. It has no role in the absorption of iron as is usually believed



### Future Lines of Work

As  $\alpha$ -cells of islets of Langerhans of the pancreas are increased in scurvy, the relation of glucagon, the  $\alpha$ -cell hormone, to vitamin C-nutrition should be studied. The specific role of ascorbic acid in hemoglobin synthesis should be determined. The percentage of glycosylated hemoglobin in the blood during the progressive stages of scurvy should be studied with a view to finding the role of insulin in the process.

### Selected Publications

1. Banerjee S, Relation of scurvy to the adrenaline content of the adrenal glands of guineapigs, *J biol Chem*, **159** (1945) 327-31.
2. Banerjee S, The effect of vitamin C on the glucose tolerance test in guineapigs, *Ann Biochem exp Med*, **3** (1943) 157-70.
3. Banerjee S, Biswas D K & Singh H D, Studies in carbohydrate metabolism in scorbutic guineapigs, *J biol Chem*, **230** (1958) 261-70.
4. Kawishwar W K, Chakrapani B & Banerjee S, Carbohydrate and lipid metabolism in scurvy—Effect of vitamin C supplement, *Indian J med Res*, **51** (1963) 488-93.
5. Bandyopadhyay S, Mukherjee A K & Banerjee S, Intestinal absorption of sugars in hypoinsulinism, *Indian J exp Biol*, **19** (1981) 916-19.
6. Sarkar A K & Banerjee S, Studies on the glucose tolerance test in scorbutic monkeys, *Indian J Physiol Pharmac*, **1** (1957) 27-29.
7. Banerjee S & Bandyopadhyay A, Plasma lipids in ascorbic acid-deficient rhesus monkeys, *Am J Physiol*, **208** (1965) 329-33.
8. Banerjee S & Ghosh P K, Effect of scurvy on hexokinase activity of tissues of guineapigs, *Proc Soc exp Biol Med*, **88** (1955) 415-16.
9. Banerjee S & Ghosh P K, Effect of insulin on glucokinase activity of tissues of scorbutic guineapigs, *J scient ind Res*, **20C** (1961) 193-94.
10. Lahiri S & Banerjee S, Carbohydrate metabolism and phosphate turnover rate in scorbutic guineapigs, *Proc Soc exp Biol Med*, **93** (1956) 557-60.
11. Banerjee S & Divakaran E R, Utilization of glucose, fructose and galactose by scorbutic guineapigs, *Am J Physiol*, **195** (1958) 291-94.
12. Banerjee S & Singh H D, Cholesterol metabolism in scorbutic guineapigs, *J biol Chem*, **233** (1958) 336-39.
13. Banerjee S & Singh H D, Metabolism of citric acid in scorbutic guineapigs, *J biol Chem*, **235** (1960) 902-5.
14. Banerjee S & Biswas D K, Urinary excretion of pyruvic acid, alphaketoglutaric acid and oxaloacetic acid in scurvy, *J biol Chem*, **234** (1959) 3094-96.
15. Banerjee S & Kawishwar W K, Studies on the operation of the tricarboxylic acid cycle in scurvy, *J biol Chem*, **234** (1959) 1347-49.
16. Banerjee S & Ghosh N C, Relation of scurvy to glucose tolerance test, liver glycogen and insulin content of pancreas of guineapigs, *J biol Chem*, **168** (1947) 207-11.
17. Bandyopadhyay S & Banerjee S, Effect of ascorbate deficiency on liver glycogen metabolism in guineapigs, *Indian J exp Biol*, **20** (1982) 44-47.
18. Bandyopadhyay S & Banerjee S, Plasma adrenaline and tissue monoamine oxidase of scorbutic guineapigs, *Indian J exp Biol*, **19** (1981) 1091-92.
19. Belavady B & Banerjee S, Metabolism of cholesterol in scorbutic guineapigs, *J biol Chem*, **209** (1954) 641-45.
20. Lahiri S & Banerjee S, Coenzyme A activity in tissues of normal and scorbutic guineapigs, *Proc Soc exp Biol Med*, **91** (1956) 583-84.
21. Banerjee S & Ghosh P K, Metabolism of acetate in scorbutic guineapigs, *Am J Physiol*, **199** (1960) 1064-66.
22. Banerjee S & Kawishwar W K, Studies on the metabolism of butyrate in scurvy, *J scient ind Res*, **20C** (1961) 141-44.
23. Banerjee S & Bandyopadhyay A, Plasma lipids in scurvy, Effect of ascorbic acid supplement and insulin treatment, *Proc Soc exp Biol Med*, **112** (1963) 372-74.
24. Banerjee S, Relation of scurvy to histological changes in the pancreas, *Nature, Lond*, **153** (1944) 344.
25. Banerjee S & Ghosh S K, Histochemical changes in tissues of guineapigs during scurvy, *Proc natn Inst Sci India*, **29B** (1968) 225-38.
26. Banerjee S, Deb C & Belavady B, Effect of scurvy on glutathione and dehydroascorbic acid in guineapig tissues, *J biol Chem*, **195** (1952) 271-76.
27. Banerjee S, Physiological role of dehydroascorbic acid, *Indian J Physiol Pharmac*, **21** (1977) 85-93.

28. Banerjee S & Chakrabarti A S, Anemia and its relation with iron metabolism in scorbutic monkeys, *Indian J med Res*, **53** (1965) 835-48.
29. Banerjee S & Nandy M, Pteroylglutamic acid nutrition in vitamin C-deficient guineapigs, *Proc Soc exp Biol Med*, **133** (1970) 151-52.
30. Chakrabarti A S & Banerjee S. Anemia in scurvy and its relation with iron metabolism, *Indian J exp Biol*, **1** (1963) 135-40.
31. Banerjee S & Chakrabarti A S, Utilization of iron by scorbutic guineapigs, *Blood*, **25** (1965) 839-44.



## J V Bhat\*

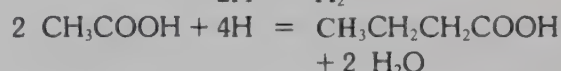
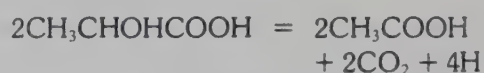
Ushering in of the twentieth century saw the blossoming in the country of two new sciences, biochemistry at the Indian Institute of Science, Bangalore, and microbiology at St. Xavier's College, Bombay. I was fortunate to be associated for two decades with each of these.

Study of microbiology of air, earth (soil) and sea became imperative, as mango—the king of fruits (notwithstanding pineapple with its crown on)—could not be contained in the can! It would huff, puff, bulge and burst and a premier factory in Bombay had to almost give up the task, though finally success was achieved—thanks to knowledge gained on the microbes causing damage. Mango and its products are now export commodities.

The significance of spore-forming bacilli in this context is well recognized. Hence, a thorough examination of fruits, vegetables, air and sea water was undertaken. Air (from different altitudes using aircraft) and soils from 28 locations spread over India (including two now in Pakistan) were examined microbiologically. Sea water was derived off Bombay coast; fruits, vegetables and other products were taken from Bombay markets. The results of this survey<sup>1</sup> revealed the presence in large numbers of several species, the dominant ones being *Bacillus subtilis* in air and soil,

*B. megaterium* in sea water and *B. cereus* in other environments. More importantly, their thermal resistance (T.D.T.) in water, brine and syrup was greater than that reported for like species in the literature; so was their resistance in preservatives. Processing of mango in the light of these findings, employing higher 'retorting' temperatures, prevented its spoilage.

Although fermentation of lactic acid to yield butyric acid was observed by Pasteur and studied by others, it remained a poorly studied subject. Attempts to search for the bacteria and ascertain the steps involved in the conversion of a 3-C into a 4-C compound resulted in the isolation of *Clostridium lacto-acetophilum* nov sp and elucidation of stepwise reactions through the use of <sup>14</sup>C and the essentiality of acetic acid as a hydrogen acceptor according to the equations<sup>3</sup>:



Unlike lactic acid, a key intermediate in the metabolism of carbohydrates by many bacteria, oxalic acid is neither formed nor easily attacked by them. A concerted effort was, therefore, made to recognize bacteria decomposing it. This resulted in the discovery of many interesting species

---

Formerly, Professor, Indian Institute of Science, Bangalore and Deputy-Director of Research, The Academy of General Education, Manipal, Kodamgallu-574197, Karnataka.

\*Since deceased.

and starting of work on the theme referred to as single carbon metabolism. *Vibrio oxaliticus* was the first species to be reported<sup>4</sup>; it was isolated from soil. Investigations on the microflora associated with earthworm led to the isolation of other species, viz., *Pseudomonas oxalaticus*, *Bacterium oxalaticum*, *Mycobacterium lacticola* and *Streptomyces*. Biochemical investigations revealed the interesting ways in which this simplest of organic compounds, the first one synthesized in the laboratory, gets metabolized, carbon dioxide associated therewith serving as the sole source of energy and carbon. In fact, its metabolism seems to represent the mid-step between heterotrophic and autotrophic ways of microbial life. The mechanism of oxalic acid metabolism was reviewed for the first time in 1958<sup>5</sup>.

Isolation of the above-mentioned species brought into focus both the earth (soil) and its ubiquitous fauna, the earthworm. That nitrogen in soil is of vital importance [notwithstanding its low levels (0.02-2.58%)] was well realized long ago. Indian soils conspicuously are poor in this element, and replenishment with any amount of organic or other matter was observed to be of no avail. Hence, an in-depth study to understand the weathering process under light, darkness, moisture, etc. and in the presence of cellulose and selected microorganisms, e.g. *Azotobacter chroococcum* and *Aerobacter aerogenes*, was undertaken. In these two species, cellulose and moisture were observed to prevent losses in nitrogen, measurable even daily<sup>6</sup>, apart from the indications the experiments presented on the possible fixation of nitrogen by bacteria.

Other investigations on these lines led to the observation that the leaves of mulberry plant (*Morus indica*), sandal (*Santalum album*) and its host beans (*Dolichos lablab*) are teeming with microbes, notably nitrogen-fixing bacteria. Through inoculation of leaves of sandal with *Azotobacter* and *Beijerinckia*, clearcut evidence was adduced on their ability to fix nitrogen thereon through analysis of root leachates of inoculated and uninoculated control plants; whereas the former contained higher amounts of the same and other amino acids (not detectable in the controls), the latter did not. Some of these significant findings were presented at a conference held in UK on the Ecology of the Leaf Surface Organism<sup>7</sup>.

Soils conjure up in the mind the panorama of flora above the surface and the crawling sensation of earthworm movements below the flora providing food, fodder, fuel, fruits and drugs and the fauna contributing to soil fertility, apart from other uses to which it is put. It was considered worthwhile to put to test some of the plants and their products for therapeutic use by methods hitherto not applied and earthworms for their associated microflora, with special reference to their role in agriculture. As mentioned earlier, oxalate decomposing bacteria represent one such reward, as it tends to explain why oxalate does not accumulate in soil notwithstanding substantial quantities added to it as plant debris.

Over 300 plant species had been in use as medicines since the days of Charaka and Sushruta, but the exact manner in which they act, as explained for antibiotics, for example, was unknown.



Discarding hundreds of plants as of no worth when they failed to kill either *Staphylococcus aureus* and/or *Escherichia coli* was, in my opinion, no proof of their impotency. New approaches were thus applied and interesting observations, though unexpected and of inestimable value, were recorded. Saffron, stylus of flower of *Crocus sativus*, for example, is held in high esteem in both Ayurvedic and Tibbi systems of medicine, but would it show antibacterial action when in fact it is rich in vitamins of the B-complex? Experiments proved it to be the richest source of riboflavin in particular, surpassing 2-3 times animal liver and dried yeasts held until then as its best sources. Rat feeding trials with 150 mg supplementation of saffron matched the need for 40 mg of pure riboflavin. Asafoetida was demonstrated to possess carminative property claimed for it on the basis of fermentation experiments carried out with a few microbial species of intestinal origin. Likewise, some other plants which failed to act on test bacterial species were shown to possess activity by adopting other experimental procedures. Through microbiological and animal experiments, *Lohasava*, an iron-containing tonic, was proved to be as good if not superior in combating iron deficiency anaemia. A good deal of the information collected was summed up in one communication<sup>8</sup>.

Coming now to earthworms, 'nature's own tillers of soil' constituting a significant group (over 1000 species exist) of living world in soil, three decades of work yielded results of far reaching import. Besides oxalate decomposers, several other species attacking cellulose and other substrates were demonstrated to be present in abundance in the intestinal tract

of the invertebrate. Of considerable interest was the presence of azotobacter, in statistically significant numbers, in the casts as compared to those encountered in the surrounding soils. Twelve excretory specimens in one trial yielded an average count of  $17,599 \pm 7,619$  cells as compared to only  $3,975 \pm 1,682$  found to be present (av for 12 soils) in the surrounding area, the statistical significance thereof being  $P = 0.1$ . This led to experiments on bacteriazation of soils to ascertain if incorporation of casts or the worms themselves in soils can mend soils by increasing azotobacterial populations. Detailed accounts of the role of earthworms in agriculture and bacteriazation experiments were published<sup>9,10</sup>. Suffice it to mention that by and large the experiments proved to be successful and as of today seed inoculation with this organism and use of fertilizers made with it have yielded greater harvests. Thus, earthworms do seem to have a vital role in agriculture.

Continued search for worms over vast stretches of land brought to notice a unique disease in *Hoplochetella suctorica* caused by a bacterium identified as *Enterobacter aerogenes*. Since no bacterial infection had prior to it been described in this animal, it drew the attention of earthworm workers all over the world, when a detailed report was presented at a conference held in UK in 1982 to commemorate the publication by Darwin of his famous book on earthworm a century ago. The disease, it may be mentioned, kills a large number of worms during August-October, i.e. soon after they have increased in numbers in monsoon season (June and July). The infection is characterized by the appearance of pinpoint depressions in the

clitellar region, spreading soon after elsewhere on the body, and assuming fairly large sized ulcerative wounds, at which stage the worms become sluggish and begin to die. A recent book<sup>11</sup> provides details of the occurrence of the disease, characterization of the aetiological agent and other related phenomena about this unique infection.

Though from earthworm to silkworm does not appear to be a far cry to laity to zoologists, it is a leap from an invertebrate to a mere insect. Since Karnataka accounts for over 70% of silk production and India is among the four top silk producing countries, attempts were made to improve the quality and yield by gaining an understanding of the nutritional needs and metabolic pattern of this unique insect economically exploited by man. A variety of techniques were evolved and a wealth of information was gathered on the worm, *Bombyx mori*, which ordinarily thrives in a singled out diet mulberry leaf, *Morus alba*. It is not possible to summarize the work which extended over two decades. As an illustration may be cited the result of one study on chloramphenicol supplementation of the diet which proved to be beneficial. *p*-Nitrobenzaldehyde, one of the products of degradation of this antibiotic, was noted to enhance the growth, whereas *p*-aminobenzoic acid, another product, inhibited growth, though this compound paradoxically is beneficial to animals and microbes in general. Very many interesting features came to be observed in the process of compounding an artificial feed, as production of mulberry leaf for feeding was proving expensive.

Since wounded soldiers were observed to get healed in sea water, it was regarded free of pathogens for man, but

investigationally sea remained unexplored until 50 odd years ago. Even then the work was confined to bacteria and yeasts were left out beyond a mere mention that they exist in the sea. Since certain foods preserved in brine were observed to undergo spoilage by yeasts, sea water was considered worthy of examination for its yeast flora. The results indeed made it clear that the yeasts in sea distinctly differ from their terrestrial counterparts in several important characters, apart from the fact that the sea flora is by and large asporogenous in contradistinction to earthly species comprising mostly sporogenous variety. In the sea, *Candida* and *Debaryomyces* were preponderant, with *C. tropicalis* and *D. hansenii* as the frequently encountered species<sup>13</sup>. Interestingly, the latter was encountered in most cases of spoilt fruits and vegetables, suggestive of their origin as sea salt.

Since jute products were high export earners and jute became scarce due to partitioning of the country, search had to be made for jute substitutes and choice fell on *Hibiscus cannabinus* and *Malachra capitata*. These, however, were poorly made raw materials, as retting thereof was not a clearly understood process. In case studies carried out in the laboratory, not only were products obtained and *M. capitata* proved as a good champion, microbiologically they made history in that the exact role of *Pseudomonas* in retting came to be revealed, as the bacteria could decompose pectic substances, a prerequisite to release of fibre. In a similar manner, the 'secret' part played by pectinolytic yeasts in the removal of the mucilage layer surrounding coffee beans of *Coffea robusta* was disclosed, this process conferring the desired quality in the export product. Other interesting features



connected with decomposition of pectic substances by microorganisms have been detailed in a review<sup>14</sup>.

Researches on marine yeasts and microorganisms decomposing pectic substances, with particular reference to plant stems, coffee and other farm produce, equipped the mind and the laboratory to understand and undertake work on coir retting, an unique industry of the country. Coir is a hardy fibre derived from the husks of coconut fruit (*Cocos nucifera*). This natural fibre may be regarded as jack of fibres when cotton is reckoned as the king and silk the queen. Coir fulfils the needs the others do not and is of multipurpose usage. India has the monopoly for retted material and it enters the markets of the world in several finished forms and even as rubberized goods. The industry is a cottage one and, like the silk industry, offers gainful employment to lakhs of people.

Retting of coir, unlike jute and other fibres which take only one to three weeks, calls for prolonged soaking of husks extending from 6 to 9 months and under certain environments even a year. Best coir is the one derived from coastal backwaters, where tides drain off and refill water that covers the husks constantly. Detailed studies<sup>15</sup> brought out the reasons for prolongation of the period as well as for the practices employed. Husks contain large quantities of polyphenolics as compared to pectic substances and these have to be leached away during tides before the decomposers of pectin can function well, though up to a period of 4 months both of them occur in an almost parallel manner. Phenolics are antibacterial and their decomposition takes place by and large by yeasts and that of pectin by

bacteria. Sea water provides ideal conditions, though retting can also be achieved in fresh water, in that the species of yeasts competent to attack polyphenolics reside in the sea and besides prevent the intense fouling witnessed in fresh water.

Enrichments with catechol and phenol clearly brought out the part played by *D. hansenii* and *Micrococcus* sp. in the removal of these antibacterial chemicals. Catechol lies in the pathway of degradation of phenolics and no organism was hitherto demonstrated to grow in it, though it was obviously involved in its metabolism. This represents the key role of marine yeasts in the process.

Pectin decomposition was observed to be brought about by both bacterial and yeast flora belonging to the genera *Achromobacter*, *Alcaligenus*, *Paracolobactrum*, *Micrococcus*, *Bacillus*, *Candida*, *Cryptococcus* and *Rhodotorula*, a few not connected with this function earlier. Isolation of *Micrococcus* sp. from phenol enrichments led to the discovery that the discoloration of the otherwise attractively golden hue of coir was due to the formation of a blackish pigment by it from phenol and identified as 3,4,3',4'-tetrahydroxydiphenyl, a product of catechol. This, of course, can be minimized, if not fully eliminated, and good price assured for the product. Several other factors also came to be known and ways understood for the reduction of retting period, apart from a thorough understanding of the chemistry and microbiology of the process of producing this precious material which alone, among the natural fibres, can resist decay under marine environments, not to emphasize its other durable and endurable qualities.

## Selected Publications

1. Bhat J V & Iyer V, *Proc Indian Acad Sci*, **42B** (1955) 325-33.
2. Iyer V & Bhat J V, *J scient ind Res*, **11B** (1952) 427-30.
3. Bhat J V & Barker H A, *J Bact*, **54** (1947) 381-91; **56** (1948) 777-79.
4. Bhat J V & Barkar H A, *J Bact*, **55** (1948) 359-68.
5. Jakoby W B & Bhat J V, *Bact Rev*, **22** (1958) 75-80.
6. Bhat J V, *J scient ind Res*, **34** (1975) 353-63.
7. Bhat J V, Limaya Kunda & Vasantharajan N V, in *Ecology of leaf surface organisms*, edited by T F Preece and C H Dickinson (Academic Press, Inc, New York) 1971, 581-95.
8. Bhat J V, *Pharmstudent (Banaras Hindu Univ)*, **13** (1962) 77-83.
9. Bhat J V & Khambata S R, Role of earthworms in agriculture, *ICAR Res Ser, Bull No 22*, New Delhi.
10. Bhat J V, in *Radioisotopes, fertilisers and cowdung gas plant* (ICAR, New Delhi) 317-21.
11. Rao B R, Karuna Sagar I & Bhat J V, in *Earthworm ecology*, edited by J E Satchell (Chapman and Hall, London) 1983, 383-91.
12. Shyamala B B & Bhat J V, in *Golden Jubilee Res Vol, Indian Inst Sci, Bangalore* (1959) 223-30.
13. Bhat J V & Kachwalla Nafisa, *Proc Indian Acad Sci*, **41B** (1955) 9-15.
14. Bhat J V, Jayashankar N P, Agate A D & Bilimoria M H, *J scient ind Res*, **27** (1956) 196-203.
15. Bhat J V, in *Coconut research and development*, edited by M N Nayar (Wiley Eastern) 1983, 259-73.



## S N Chatterjee

### (1) Microbiology of *Vibrio cholerae* and Cholera Bacteriophages

The modern techniques of molecular biology, cell biology and microbiology were applied for the first time in laying the foundations of our knowledge of the structure and function of *Vibrio cholerae* cell and also of the cholera bacteriophages. A novel excretory mechanism concerning actively growing vibrios was discovered; it was subsequently confirmed by many other investigators and will go a long way in explaining the mechanism of release of somatic antigens and cholera toxin by the actively growing vibrios. The major contributions of Chatterjee are highlighted below.

#### (i) Detection of atypical fimbriae (pilli) on *V. cholerae* cells

(a) These fimbriae detected for the first time are different from the already known 6/7 types of fimbriae (pilli) characterized by Duguid (UK) and Brinton (USA).

(b) The ultrastructure of these fimbriae was documented for the first time.

(c) This finding explained satisfactorily the haemagglutinating and pellicle forming properties of the vibrios.

#### (ii) Discovery and visualization of a novel excretory mechanism of young *V. cholerae* cells

(a) The involvement of cell wall in this excretory process and the association of toxin-like particles with the excreted blebs were elucidated.

(b) The mechanism of release of somatic antigens by the actively growing vibrios was explained for the first time.

(c) The biochemistry and the ultrastructure of the excretion process were elaborated.

(d) The mechanism of release of cholera toxin, a proteinous molecule of fairly high molecular weight, through the barriers of plasma membrane and cell wall, was visualized.

#### (iii) Characterization of cholera bacteriophages using modern phage techniques

(a) The ultrastructure of cholera phages was recorded for the first time.

(b) The molecular mechanisms of infection and intracellular multiplication of cholera phages were elucidated.

(c) Interaction of these phages with physical and chemical agents was elaborated.

(d) The morphology and serological classifications of these phages were equated for the first time.

(e) The molecular basis of differentiation of *V. cholerae* (classical) and *V. cholerae* (El Tor) strains by Gr IV phage was explained.

(f) The necessity for electron microscopic identity control of these phages was established.

(iv) *Other important contributions*

(a) Flagellar ultrastructure and mode of attachment to the vibrio cell were documented for the first time.

(b) The ultrastructural features of *Vibrio cholerae* cells were also documented for the first time.

(c) Trilamellar nature of the structure of *V. cholerae* cell wall was revealed and the molecular basis of the differences in structure and the function of the cell wall of cholera and El Tor vibrios was elaborated.

(d) The mechanism of release of somatic antigens by the vibrios in glucose-saline medium was visualized and explained for the first time.

(e) The first stage of characterization of *Vibrio cholerae* genome DNA was documented in a series of publications.

(f) The ultraviolet inactivation characteristics of the vibrios was documented for the first time. Genetic differences between the different vibrio biotypes were established.

**(3) Discovery of a New Inhibitor of DNA Biosynthesis, Furazolidone, and Elucidation of the Molecular Mechanism of Its Action**

Drugs, including antibiotics, played a key role in the development of the subject of molecular biology by virtue of their

ability to interfere selectively in the synthesis of cellular macromolecules. Although furazolidone was known to have an inhibitory effect on *V. cholerae* and some other microbial cells from clinical and laboratory trials, its mode of action, particularly at the molecular level, was not known. The molecular mechanism of action of this drug has been worked out for the first time with outstanding details and recorded in a large number of publications in internationally reputed journals. The significant and novel findings are highlighted below.

(i) Furazolidone inhibits DNA biosynthesis and cell division, leading to filamentation of the cells.

(ii) Bacteriophage DNA synthesis in the infected host is at least 10 times more sensitive to this inhibitory action of the drug. Phage receptors on the cell surface are unaffected.

(iii) Furazolidone undergoes metabolic transformation within the cell and then produces *in vivo* inter-strand crosslinks in DNA.

(a) This explains for the first time the mechanism of inhibition of DNA biosynthesis by it.

(b) Crosslinking imparts thermal stabilization to DNA.

(c) Crosslinking makes the DNA reversibly bihelical.

(d) The number of inter-strand crosslinks per *V. cholerae* genome DNA has been analysed and estimated mathematically.

(iv) Furazolidone induced DNA lesions can be repaired. Both excisional and recombinational repair mechanisms have



been shown to be involved in removing such lesions.

(v) Furazolidone is mutagenic and leads to prophage induction. Is furazolidone carcinogenic? The relevant findings are :

(a) It caused mutation of streptomycin sensitive cells to streptomycin resistant cells.

(b) It led to prophage induction in both *V. cholerae* and *E. coli* K-12 cells.

(vi) The following photobiological properties of furazolidone have been identified :

(a) Furazolidone binds with DNA *in vitro* by an intercalative process. This is different from the *in vivo* nature of binding.

(b) Binding stoichiometry has been worked out in detail.

(c) Furazolidone leads to the photodynamic inactivation of bacterial viruses *in vitro*.

(d) Furazolidone also protects these viruses from ultraviolet inactivation.

(e) *In vitro* binding of furazolidone with DNA has preference for A-T base pairs, leading to thermal stabilization of DNA and inhibition of digestion of DNA by DNase.

These studies have provided a new biochemical tool (inhibitor of DNA synthesis) to the molecular biologists. Since furazolidone is being used in the treatment of clinical cases, elucidation of the molecular mechanism of its action will have not only fundamental but also applied value. Knowledge about photobiological properties of furazolidone is likely to open a new chapter in the therapeutic use of the drug, viz., photo-chemotherapy of surface infections, including viral infections.

### **(3) Discovery of Lipid Peroxidation as the Molecular Basis of Membrane Damage by Ultraviolet Light and Sunlight**

The significant findings are highlighted below.

(i) That membranes could be the primary target of far and near ultraviolet light and sunlight has been conclusively proved for the first time.

(ii) These radiations cause lipid peroxidation in membranes through a free radical mediated reaction.

(iii) The different products of lipid peroxidation, viz., diene conjugates, lipid hydroperoxides and malondialdehyde, have been detected and estimated.

(iv) Dose and dose rate effects have been analysed and the inverse dose rate effect revealed.

(v) Lipid peroxidation makes the membrane leaky. The effects of radiation dose and dose rates on leakiness of the membrane have been analysed.

(vi) Thoroughly characterized model membrane systems, e.g., liposomes, have been used in such studies to eliminate interference by other agents.

Malondialdehyde and other lipid peroxidation products are suspected mutagenic and carcinogenic agents. The above findings provide for the first time the molecular basis of the causation of skin cancer by exposure to sunlight and ultraviolet light.

### **(4) Electron Microscopic Visualization and Elucidation of the Structures of Biomolecules**

*Haemoglobin* : Photograph of an individual molecule of haemoglobin was

recorded for the first time by electron microscopy in 1958-1960. The electron microscopic model of this molecule was in good agreement with the X-ray model derived by Max Perutz a few years earlier.

**Serum albumin** The structure of the serum albumin molecule had been the subject of controversy for several years. RC Valentine of the National Institute for Medical Research, Mill Hill, UK, attempted to photograph the molecule by electron microscope but admitted failure [*Nature, Lond*, **184** (1959) 1838]. In this context, the individual molecules of serum albumin were photographed successfully by electron microscopy. This study provided the first direct evidence of the presence of morphological subunits in this molecule, although each molecule was made of only one polypeptide chain.

**In situ polyribosomal structures :** Polyribosomal structures were visualized for the first time by Werner, Rich and Hall in 1962. But those were the pictures of the polyribosomes after these were isolated from the cell and purified by differential centrifugation. Visualization for the first time of the polyribosomes within the cells by ultrathin sectioning and electron microscopy was achieved by our studies. Even the connecting mRNA threads could be discerned in the electron micrographs recorded.

## Selected Publications

1. Chatterjee S N, Sadhukhan P & Chatterjee J B, Electron microscopic studies on the haemoglobin

molecules, *J Biophys Biochem Cytol*, **10** (1961) 113-17.

2. Chatterjee Amala & Chatterjee S N, Electron microscopic studies of the serum albumin molecules, *J molec Biol*, **11** (1956) 432-37.
3. Chatterjee S N & Das J, Electron microscopic observations on the excretion of cell wall material by *Vibrio cholerae*, *J gen Microbiol*, **40** (1967) 1-11.
4. Das J & Chatterjee S N, Morphological changes of *Vibrio cholerae* cells in glucose saline, *J gen Microbiol*, **54** (1968) 445-50.
5. Chatterjee S N & Sen Gupta P C, Polyribosomes in thin section of *Leishmania donovani*, *Z Naturf*, **25b** (1970) 232-34.
6. Raichaudhuri C, Chatterjee S N & Maiti M, Effects of furazolidone on the macromolecular synthesis and morphology of *Vibrio cholerae* cells, *Biochim biophys Acta*, **222** (1970) 637-46.
7. Chatterjee S N & Maiti M, Effects of furazolidone on the infection of *Vibrio cholerae* by the phage  $\phi$  149, *J Virol*, **11** (1973) 872-78.
8. Mandal T K, Ghosh Srabani & Chatterjee S N, Effect of ultraviolet radiation on the liposomal membrane, *Int J Radiat Biol*, **33** (1978) 75-79.
9. Mandal T K & Chatterjee S N, Ultraviolet and sunlight induced lipid peroxidation in liposomal membrane, *Radiat Res*, **83** (1980) 290-302.
10. Banerjee S K & Chatterjee S N, Radiomimetic property of furazolidone and the caffeine enhancement of its lethal action on the vibrios, *Chem Biol Interact*, **37** (1981) 321-35.
11. Chatterjee S N, Banerjee S K, Pal A K & Basak Jayasri, DNA damage, prophage induction and mutation by furazolidone, *Chem Biol Interact*, **45** (1983) 315-26.
12. Agarwal S & Chatterjee S N, Peroxidation of the dried thin film of lipid by high energy alpha particles from a cyclotron, *Radiat Res*, **100** (1984) 257-63.
13. Chatterjee S N, Radiations, biomembranes and cancer, *Indian J Phys*, **58A** (1984) 35-48.
14. Chatterjee S N & Maiti M, Vibriophages and vibriocins: Physical, chemical and biological properties, *Advances in virus research*, Vol 29 (Academic Press Inc, New York) 1984, 264-312.
15. Pal A K & Chatterjee S N, Prophage induction by furazolidone, *Mutat Res*, **156** (1985) 69-75.



## S R K Chopra

The research career of Chopra, extending over three decades, has encompassed various aspects of physical anthropology, mainly primate osteology, including craniology and osteometry, hominoid palaeoprimatology, and human growth. A synoptic outline of the type of research work done by him is given below.

While at Zurich, Switzerland (1953-55), he conducted research on cranial suture closure in the old world and new world monkeys. The findings<sup>1</sup> provided new information on suture closure in relation to the physiological age of monkeys. This contribution has gained significance, as the data have been compared with the corresponding data for anthropoid apes and man. It finds prominent reference in standard works on primates.

At the University of Birmingham (1955-58), under a grant from the Wenner-Gren Foundation for Anthropological Research, Inc., New York, Chopra designed a pelvimeter<sup>2</sup> for the measurement of angles of torsion on the pelvis and other bones of primates, including man, which was till then impossible to achieve with the conventional osteometric instruments. Subsequently, with the use of this instrument, he worked on the angle of pelvic torsion in living primates, and the South African Australopithecine innominate bone<sup>3</sup>. Some of these results have been discussed in respect of the

problem of erect posture in Australopithecines in various research papers and reports.

At the Panjabi University from 1967 onwards, Chopra and his colleagues have been pursuing a programme of major research investigations relating to the problem of human origin and development of quarriable sites for the search of remains of early man and his relatives in the Mio-Pliocene strata of the Sivalik Hills. Under this programme, they have recovered a large collection of vertebrate fossil remains, including those of primates, which can be rated as one of the best anywhere. One of the primate fossil remains is a lower jaw, perhaps the most complete of any fossil anthropoid found in the Sivaliks. This is now the famous *Gigantopithecus bilaspurensis*, the scientific details of which were presented at the second international congress of primatology, Atlanta, Georgia, USA (1968) and subsequently published<sup>4</sup>. Besides various research reports and papers, references to *G. bilaspurensis* have been made in various text and reference books on palaeoprimatology and palaeoanthropology. This work was also summarized in Chopra's presidential address to the Section of Anthropology and Archaeology of the 61st session of the Indian Science Congress Association<sup>5</sup>.

---

Vice-Chancellor, Kurukshetra University, Kurukshetra, Haryana.

Of various other Mio-Pliocene primate fossil remains recovered during many expeditions to Sivaliks, Chopra presented scientific details on some dryopithecine material at the fifth international Congress of Primatology held at Nagoya, Japan in 1974. These were also published in "Contemporary Primatology"<sup>6</sup>. The evidence provided by these remains indicates a remarkable variety of dryopithecine fauna varying considerably in general size and dimensions of their teeth, refuting to a great extent the general belief that the fossil ape species in the Haritalyangar area in India are not diversified. [See, for instance, Simons, EL & Pilbeam, D R in "The Functional and Evolutionary Biology of Primates", edited by R Tuttle (Aldine-Atherton, Inc, New York) 1972, 56-62.]

Other achievements from the expeditions of Chopra and colleagues to the Sivaliks have been the discovery of fossils of a gibbon-like primate (*Pliopithecus krishnaii*) in the Miocene beds, and of the orang-like primate (*Sivasimia chinjensis*) from the chinjis. These are the first reports on the presence of such fossils in the Sivaliks, the preliminary scientific announcements of which were made at various national and international conferences. A number of papers on fossil lower primates, i.e. tree-shrews, adapids and Indraloris were published<sup>7-15</sup>.

In recognition of his contributions to anthropological researches, the Ethnographic & Folk Culture Society, Lucknow, invited him to deliver the coveted D N Majumdar Memorial Lectures (1976).

In respect of human growth and physique, research was conducted under a

scheme on "Anthropological variability in Himalayan populations living under varied altitude environments" which formed a part of a research report published in *Human adaptability—An international compendium* (Cambridge University Press).

At the invitation of Prof A B Chairelli, Florence University, President of the eighth congress of the International Primatological Society, Chopra delivered a main lecture at the congress held in July 1980 at Florence. Another lecture "Significance of New Hominoid Discoveries from the Sivalik Hills of India" was delivered at a Pre-Congress Symposium entitled "Miocene Hominoids and new interpretation of Ape and Human Ancestry". This has been included as a chapter in the book "New Interpretation of Ape and Human Ancestry", edited by R L Ciochon and R S Corruccini (Plenum Publishing Corporation, New York, USA).

In 1980-81, Chopra was invited to deliver the University Grants Commission's national lectures. Recently, he was invited by the Palaeontological Society of India to deliver the second M R Sahni Memorial Lecture for the year 1984 at Lucknow.

### Selected Publications

1. Chopra S R K, The cranial suture closure in monkeys, *Proc zool Soc, Lond*, **128** (1957) 67.
2. Chopra S R K, A 'pelvimeter' for orientation and measurements of the innominate bone, *Man, Lond*, **58** (1958) 171.
3. Chopra S R K, The innominate bone of the *Australopithecus* and the problem of erect posture, *Bibl Primat* (S Karger, Basel & New York) **1** (1962) 93.
4. Chopra S R K & Simons E L, A preliminary announcement of a new *Gigantopithecus* species from India, in *Recent advances in primatology*, edited by H O Hober (S Karger, Basel & New York) 1969.



5. Chopra S R K, Palaeoprimatological studies in India with reference to recent finds in the Sivaliks, *Presidential Address, Section of Anthropology & Archaeology, 61st Session of the Indian Science Congress, Nagpur, 1974.*
6. Chopra S R K & Kaul S, New fossil *Dryopithecus* material from the Nagri Beds at Haritalyangar (HP) India, in *Contemporary primatology*, Vol 2, edited by S Kondo, M Kawai & A Ehara (S Karger, Basel & New York) 1975.
7. Chopra S R K, New fossil evidence on the evolution of Hominoidea in the Sivaliks and its bearing on the problem of evolution of Early Man in India, *J hum Evol*, **7** (1977) 3.
8. Chopra S R K, Palaeontological evidence bearing on the problem of human origins in North-west India, in *Early Man in North-West India*, edited by S R K Chopra (Allied Publishers Pvt Ltd, Bombay) 1979.
9. Chopra S R K & Vasishat R N, Sivalik fossil tree shrews from Haritalyangar, India, *Nature, Lond*, **281** (1979) 214.
10. Chopra S R K & Kaul S, A new species of *Pliopithecus* from the Indian Sivaliks, *J hum Evol*, **8** (1979) 475.
11. Chopra S R K & Vasishat R N, A new Mio-Pliocene *Indraloris* (Primate) material with comments on the taxonomic status of *Sivanasua* (Carnivore) from the Sivaliks of the Indian sub-continent, *J hum Evol*, **9** (1980) 129.
12. Chopra S R K & Vasishat R N, Premiere indication de la presence dans le Mio-Pliocene des Sivaliks de l'Inde d'un Primate adapide' *Indoadapis shivani*, nov gen, nov sp, *C r Acad Sci, Paris*, **290** (1980) 511.
13. Chopra S R K, Kaul S & Pathak R K, Morphometric affinities in innominate bones of Old World Primates including man, *J hum Evol*, **11** (1982) 1.
14. Chopra S R K, Significance of recent Hominoid discoveries from the Sivalik hills of India, in *New interpretations of ape and human ancestry*, edited by R L Ciochon & R S Corruccini (Plenum Press, New York) 1983, 539.
15. Chopra S R K & Gaur R, Taphonomy, fauna, environment and ecology of Upper Sivaliks (Pliocene-Pleistocene) near Chandigarh, India, *Nature, Lond*, **308** (1984) 353.
16. Chopra S R K, *Early man in North-West India* (Allied Publishers Pvt Ltd, Bombay) 1979.

## A B Chowdhury

Chowdhury is a research scientist of established repute in the field of medical parasitology, specially immunology and epidemiology. Many of his research contributions are of outstanding merit and have brought him international acclaim. Indeed, he has contributed substantially to raising the standard of parasitology research in India. His research yield bears the stamp of a rare combination of fundamental and applied studies ranging widely from bed-side clinical research and field studies on one hand to the use of electron microscope, radio-isotopes, immunofluorescence and immunodiffusion on the other.

He made pioneering studies to determine the microchemical composition of human parasites using histochemical methods and disclosed functional responsibilities of the ultrastructural components of many parasites with the help of electron microscopy. These observations have been recognized to be of great help in understanding the biochemical and physiological activities of the parasites, including their disease producing abilities. These have explained the mode of drug action in some parasitic diseases and have enlarged the scope for improving the strategy of chemotherapy.

His studies on different facets of host-parasite relationship, specially the way

quality and quantity of host reaction, including the immune reactions in response to parasitic infections, are determined and also their geographic variations, have led to a clearer understanding of parasitic pathogenicity. The application of new immunologic techniques by him constitutes a significant advance in the immunodiagnosis of parasitic diseases. His observations indicated for the first time the superiority of antigen from homologous source and the limitations of heterologous antigen in the immunodiagnosis of parasitic diseases. He has provided the much-needed information about the quality of immune status in visceral leishmaniasis, its modulation and relationship with the severity of clinical condition and drug-induced clinical response.

His studies on the biology of parasites, their growth requirements, response to the physico-chemical composition of the environment, and factors influencing the efficiency of transmitting vectors are of considerable importance in the understanding of the transmission dynamics of parasitic diseases. He has recorded valuable observations on the effects of X-ray irradiation on the developing parasites and his success in tagging the parasites with radioactive isotopes has opened up new vistas for the



exploration of unsolved problems relating to parasitic diseases.

His observations on the animal parasites causing human diseases, parasitic zoonosis, are quite revealing, particularly in the study of the aetiopathogenesis of diseases, viz., tropical eosinophilia, eosinophilic meningo-encephalitis and hydatid disease.

He quantified the dimension of public-health problem due to parasitic infections in rural West Bengal for the first time with the help of the statistically appropriate population sampling procedure. His studies on the population biology of hookworms and the regulatory mechanism of their natural abundance in a given community are of great importance. He and his colleagues recorded for the first time the presence in man of hypobiotic hookworms with arrested development as an adaptation to a seasonally unfavourable external environment. This has revolutionized the epidemiological concept and the strategy for control of the disease.

He is one of the few who have carried out extensive scientifically designed clinical trials with a large number of anthelmintics, leading to their proper evaluation and formulation of the most effective dosage schedules. Indeed, he is considered an authority in this area, as will be borne out by the fact that he has been invited to author the section on parasitic diseases in a number of books dealing with treatment of diseases, published from outside India.

His works are quoted profusely in contemporary articles, reviews, monographs and books in his field all over the world. He has been invited to participate in numerous conferences, symposia and seminars, national and

international, and to preside over many of these. He has also been elected member and office-bearer, including president and vice-president, of many scientific bodies and organisations, national and international. He has been the Vice-President of the World Federation of Parasitologists.

### Selected Publications

1. Chowdhury A B, Das Gupta B & Ray H N, "Kernochtrot" or nuclear-fast red in the histochemical detection of calcareous corpuscle in *Taenia saginata*, *Nature, Lond*, **176** (1955) 701.
2. Chowdhury A B & Browne H G, The ultrastructure of the intestinal wall of *Ancylostoma caninum*, *J Parasit*, **45** (1959) 241.
3. Chowdhury A B, Kean B H & Browne H G, Inoculation of helminth eggs into the animal eyes, *Am J Path*, **46** (1960) 726.
4. Chowdhury A B, Das Gupta B & Ray H N, On the nature and structure of the calcareous corpuscles in *Taenia saginata*, *Parasitology*, **52** (1962) 153.
5. Browne H G, Chowdhury A B & Lipscomb L, Further studies on the ultrastructure and histochemistry of the intestinal wall of *Ancylostoma caninum*, *J Parasit*, **51** (1965) 385.
6. Schad G A & Chowdhury A B, *Trichinella spiralis* in India. I. Its history in India, rediscovery in Calcutta, and the ecology of its maintenance in nature, *Trans R Soc trop Med Hyg*, **61** (1967) 244.
7. Schad G A, Nundy S, Chowdhury A B & Bandyopadhyay A K, *Trichinella spiralis* in India. II. Characterisation of a strain isolated from a Civet cat in Calcutta, *Trans R Soc trop Med Hyg*, **61** (1967) 249.
8. Chowdhury A B & Schiller E L, A survey of parasitic infections in a rural community near Calcutta, *Am J Epid*, **87** (1968) 299.
9. Chowdhury A B, Schad G A & Schiller E L, The prevalence of intestinal helminths in religious groups of a rural community near Calcutta, *Am J Epid*, **87** (1968) 315.
10. Higeshi G I & Chowdhury A B, Immunoglobulins and complement in sera from patients with various parasitic infections, *Indian J med Res*, **59** (1971) 382.

11. Chowdhury A B & Schad G A, *Ancylostoma ceylanicum*: A parasite of man in Calcutta and environs, *Am J trop Med Hyg*, **21** (1972) 300.
12. Kochar V K, Chowdhury A B, Dean C G & Nawalinski T, Human factors in the regulation of parasitic infections: Cultural ecology of hookworm populations in rural West Bengal, in *Medical anthropology*, edited by FX Grotting & HB Haley (Mouton Publishers, The Hague, Netherlands) 1973, 287-312.
13. Schad G A, Chowdhury A B, Dean C G, Kochar V K, Nawalinski T, Thomas J & Tonascia J A, Arrested development in human hookworm infections: An adaptation to a seasonally unfavourable external environment, *Science*, **180** (1973) 502.
14. Schad G A, Soulsby E J L, Chowdhury A B & Gilles H M, Epidemiological and serological studies of hookworm infection in endemic areas in India and West Africa, in *Nuclear techniques in helminthology research* (Int Atomic Energy Agency, Vienna) 1975, 41.
15. Nawalinski T, Schad G A & Chowdhury A B, Population biology of hookworms in children in rural West Bengal. I. General parasitological considerations, *Am J trop Med Hyg*, **27**(6) (1978) 1152-61.
16. Nawalinski T, Schad G A & Chowdhury A B, Population biology of hookworms in children in rural West Bengal. II. Acquisition and loss of hookworms, *Am J trop Med Hyg*, **27**(6) (1978) 1162-73.
17. Chowdhury A B, Importance of nematodes on the Indian subcontinent, *Health Policies in Developing Countries, The Royal Society of Medicine—International Congress and Symposium Series No. 24*, 1980, 97.
18. Chowdhury A B, Parasitic infections of the human alimentary tract, in *Review of Advances in Parasitology*, edited by W Slusarski, Warsaw, 1981, 231-41.
19. Nandy A & Chowdhury A B, Lymphadenopathy in Indian Kala-azar, *Ann trop Med Parasit*, **78**(3) (1984) 331.
20. Hati A K, Nandy A & Chowdhury A B, An epidemic outbreak of Kala-azar in a district in West Bengal, India, WHO/VBC/85, 911, WHO/LEISH/85.22.



## C R Das Gupta

Systematic study of haematology in India was initiated by Dr L E Napier in association with Das Gupta at the School of Tropical Medicine, Calcutta, in 1932. The study was undertaken to find out the relative incidence of different types of anaemia in the urban and rural population and to find out its association, if any, with any known causes of anaemia, particularly hookworm and malaria and to suggest appropriate treatment as also measures for their prevention.

An impetus to haematological study was provided by the then IRFA (Indian Research Fund Association), which is now designated as ICMR (Indian Council of Medical Research), through the establishment of two Haematological Units in 1949. One of the units was established under Das Gupta at the School of Tropical Medicine, Calcutta and the other under Dr J C Patel at the KEM Hospital, Bombay. While the unit at Bombay ceased to function from 1952, the one at Calcutta developed into an excellent research centre under the guidance of Das Gupta. The establishment of the Indian Society of Haematology in 1969 which meets once a year provided a forum for the discussion of haematological problems of the country at national level and to promote and foster the exchange of ideas relating to haematological disorders in the country. In his inaugural address, the eminent

pathologist, the late Dr V R Khanolkar, paid eloquent tribute to Das Gupta, the first President of the Society, who according to him, was the first among Indians who had taken haematological studies as a speciality. The fourth Congress of the Asian and Pacific Society of Haematology held in 1967 under the Presidentship of Das Gupta was sponsored by the Indian Society of Haematology. The sixth Asian-Pacific meeting of the International Society of Haematology has also been sponsored by the Indian Society of Haematology and is due to meet in Bombay during 7-10 December 1987 under the joint chairmanship of Das Gupta and J C Patel.

### Haematological Norms of Indians

In carrying out haematological investigations, Das Gupta and his associates were faced with the absence of any normal standards of the Indian population among whom they were working. To counteract this deficiency, they set out to work out haematological norms of people residing in Bengal and other places. Das Gupta and his associates worked out the haematological norms in men of different status; women both non-pregnant and pregnant and children<sup>1-4</sup>. Das Gupta also worked out the haematological norms of men and women living at high altitude<sup>5</sup>, as also of

---

Formerly, Head, Department of Haematology, School of Tropical Medicine, Calcutta; *Residence* : 1/25, Prince Golam Mohammad Road, Kalighat, Calcutta-700026.

the purely vegetarian people of South India who lived in Calcutta. Normal haematological values of males of good economic status were almost the same as those of Europeans and Americans, but the normal values of females of good economic status were definitely lower, while those of lower economic group and of pregnant females were much lower than those of Europeans and Americans. The haematological values of people living at high altitude, including those of labourers, were much higher than those of people living in the plains.

### **Haematological Techniques**

Correct techniques are essential prerequisites for proper diagnosis of any haematological disorder and Das Gupta tried to follow them as assiduously as possible. For the most part he followed the techniques which were thoroughly tested by laboratory workers in every part of the world and were generally accepted as sound and accurate. But in a few instances, modifications were introduced to suit local conditions which seemed to give better results. Das Gupta, in collaboration with Dr Napier, wrote a book on haematological technique in 1941<sup>6</sup>. An enlarged revised edition was published by Das Gupta in 1977<sup>7</sup> in which a number of new techniques were incorporated.

### **Iron Deficiency Anaemia**

Though various types of nutritional anaemias were seen prevalent in the country at that time, the commonest type of nutritional anaemia seen was iron deficiency anaemia (IDA). IDA was seen more frequently in children and women. According to Das Gupta, IDA in Indians was due to poor intake of iron in the food;

faulty way of cooking; and loss of blood from various parts of the body. In the case of women it was due to excessive bleeding through menstruation and repeated pregnancy.

As in all cases of nutritional anaemias, there is no significant diagnostic feature by which the condition can be diagnosed clinically; the diagnosis is by blood and bone-marrow examination. The blood picture is microcytic hypochromic and the bone marrow is hypercellular and normoblastic; the normoblasts may be as high as 60-70% of the total nucleated cells. Many of the normoblasts are deformed and without adequate haemoglobin content. Most cases of IDA can be cured with therapeutic doses of iron. Das Gupta obtained excellent results with ferrous sulphate in doses of 12-18 grains a day. From a comparative study with ferrous sulphate and ferrous gluconate, Das Gupta showed that there was no difference in the curative effect of the two where the same dose of elemental iron was given. Das Gupta recommended the use of ferrous sulphate, as it was much cheaper. He also showed that copper by itself has no role in the cure of IDA.

### **Idiopathic Hypochromic Anaemia**

Idiopathic hypochromic anaemia which was seen in a large number of people in Europe and the United States was very rare in India. In his long experience, Das Gupta had seen only one case of this anaemia with koilonychia and that too in an European lady residing in India<sup>8</sup>. The patient was completely cured with oral iron.

### **Nutritional Macrocytic Anaemia**

In India, nutritional macrocytic anaemia (NMA) has been reported mostly from



Agra, Assam, Bengal, Bihar, Bombay and Madras. Though the incidence of NMA is much lower than that of IDA, much more work has been published on the subject on account of high mortality and morbidity.

NMA has been reported at all ages. However, the disease is most common between the ages of 20 and 30 years. Except in pregnant women, the incidence is higher in the males. The condition is mostly seen in the poor classes, but is also quite common in the middle income group and is only occasionally seen in the rich. It is mostly seen in the Hindus and Muslims who form the bulk of the total population; it has also been reported in Indian Christians, Jews and Anglo-Indians, but never in the European residents in the country.

Das Gupta has shown that the diseased condition is relieved, partially or wholly, with liver extract, given orally<sup>9</sup> or parenterally as crude<sup>10</sup> or as refined<sup>11</sup>, folic acid<sup>12,13</sup>, vitamin B12<sup>14,15</sup> and citrovorum factor<sup>16</sup>. Response to crude liver extract has been uniformly good, while the response to folic acid and vitamin B12 has been found to vary in different parts of the country; this has been attributed to the varying dietary habits of the people in different regions. Partial response to hospital diet was found in some cases, which, however, required treatment with anti-megaloblastic drugs for complete recovery. Unlike pernicious anaemia, cases of NMA do not show any neurological changes and do not require any maintenance treatment, but in some cases relapse may occur<sup>17</sup>. According to Das Gupta, dietary inadequacy and/or hyper-splenism, appear to be the contributory factors for the precipitation of relapse. The state of gastric acidity does

not appear to influence relapse in NMA and achlorhydria when present is usually reversible; gastric acidity returns with the improvement of anaemic condition<sup>14</sup>.

### **Dimorphic Anaemia**

Nutritional anaemia due to deficiency of more than one nutritional factors is not uncommon; deficiency of protein is seen in many cases of NMA. For the past many years, deficiency of iron along with that of folic acid and/or vitamin B12 is being seen in large numbers. The name dimorphic anaemia was suggested by Trowell in 1942 for such a condition on account of the presence of two populations of cells, macrocytes and microcytes, in the blood smear. Detailed studies of this condition were made by Chatterjee and Das Gupta in 1953<sup>18</sup>. With increasing deficiency of essential foodstuffs all over the country, dimorphic anaemia is being seen in very large numbers all over India. In a majority of cases, there is considerable deficiency of both the factors, but deficiency of one factor may predominate and the characteristic macrocytic hypochromic blood picture may not be seen; the blood picture may be macrocytic orthochromic or normocytic hypochromic, depending on the extent of deficiency of the nutritional factors. Irrespective of the blood picture, the bone-marrow usually shows a dimorphic picture; megaloblasts are found side by side with malformed normoblasts with incomplete haemoglobinisation, while in a few cases hypochromic megaloblasts, i.e. megaloblasts with non-haemoglobinised cytoplasm, have been recorded.

### **Anaemia in Pregnancy**

All types of nutritional anaemias are seen in very large numbers in pregnant women and both maternal and infantile

mortality rate from anaemia is very high. Though a considerable amount of data have accumulated on the various aspects, the information available on the subject has not been well correlated. The anaemia when detected in a pregnant woman can be cured with judicious management. Though these facts were emphasized by Napier and Neal-Edwards, Pandit and very elaborately by Das Gupta<sup>19</sup> and a number of recommendations made for the prevention and cure of anaemia in pregnant women, no serious attempt has been made to implement those recommendations and maternal mortality and morbidity continue to be very high.

### Hookworm Anaemia

Hookworm anaemia is widely prevalent in many parts of India, particularly in Assam, Bengal, Bihar, Madras, Madhya Pradesh, Travancore and Coorg. The anaemia is usually due to deficiency of iron. In hookworm anaemia, the degree of hookworm infestation and the duration of the infestation appear to be the major causative factors. Rhoads *et al.* reported that people consuming meat did not show any anaemia even when the infestation with hookworm was heavy. Napier and Das Gupta had also shown that high protein diet alone would not improve the anaemia in hookworm disease but whether people consuming much meat would develop anaemia if infected with hookworm is still to be corroborated. Napier, Das Gupta and Mazumder<sup>20</sup> had conclusively shown that treatment with iron, two courses of iron given orally, raised the blood picture to normal level even in very anaemic patients. Later deworming with any potent anthelmintic, e.g. tetrachlorethylene, will help to maintain the blood picture at the normal

level. In a few cases, a third course of treatment with iron may be required. In some cases, medication with iron may have to be continued even after improvement of blood picture to make good the depleted iron stores in the body, particularly in cases suffering from anaemia for a very long time.

### Transfusion of Blood in Nutritional Anaemias

Das Gupta found transfusion of blood to be a valuable adjunct in the treatment of NMA with very low red cell count. Repeated transfusions of a small quantity of blood, say 200-250 ml at a time, was found to give better results than massive transfusion of 500-1,000 ml of blood at a time. Das Gupta did not notice higher rate of improvement in the cases having transfusion of blood given along with anti-megaloblastic drugs compared with the cases receiving anti-megaloblastic drug alone. It must, however, be admitted that judicious transfusion of blood always helps to improve the general condition of the patients immensely.

Das Gupta noted that in the treatment of dimorphic anaemias, judicious transfusion of blood has almost the same effect as in the treatment of NMA. No transfusion of blood is required in the treatment of IDA even when the haemoglobin value is very low; iron and iron alone will help to improve the blood picture in these cases. Hookworm anaemia cases also do not require any transfusion of blood<sup>21</sup>.

In cases of anaemia in pregnancy, repeated transfusion of small amounts of blood helps in improvement of the blood picture, particularly in the cases which are seen in the later stage of pregnancy. Thus,



transfusion of blood is a valuable adjunct in the treatment of the cases mentioned above when used in conjunction with appropriate haematinics.

### Selected Publications

1. Napier L R & Das Gupta C R, Haematological studies in Indians: Part I. Haemoglobin estimation methods, *Indian J med Res*, **22** (1938) 89.
2. Napier L R & Das Gupta C R, Haematological studies in Indians: Part II. Normal Bengali town population, *Indian J med Res*, **23** (1935) 305.
3. Napier L R & Das Gupta C R, Haematological studies in Indians: Part III. Normal standards for tea garden cooli population, *Indian J med Res*, **23** (1935) 311.
4. Napier L R & Das Gupta C R, Haematological studies in Indians: Part XII. Haemoglobin standards in children and adolescents, *Indian J med Res*, **28** (1940) 267.
5. Das Gupta C R, Blood picture of Indians at high altitude, *Indian med Gaz*, **86** (1952) 11.
6. Napier L R & Das Gupta C R, *Haematological Technique* (Thacker Spink & Co, Calcutta), 1st Edn, 1941.
7. Napier L R & Das Gupta C R, *Haematological Technique* (Revised by C R Das Gupta) Enlarged 4th Edn, 1977.
8. Das Gupta C R, Idiopathic hypochromic anaemia with a case note, *Indian med Gaz*, **75** (1940) 193.
9. Das Gupta C R, Ganguli S & Chatterjea J B, Proteolysed liver extract in the treatment of tropical macrocytic anaemia, *Indian med Gaz*, **81** (1946) 122.
10. Das Gupta C R, Indian made liver extract in the treatment of nutritional macrocytic anaemia, *Indian med Gaz*, **78** (1943) 35.
11. Napier L E, Das Gupta C R, Chaudhari R N, Sen G N, Raichaudhuri M N, Sengupta P C & Mazumder D N, Anahaemin in tropical macrocytic anaemia, *Indian med Gaz*, **73** (1938) 283.
12. Das Gupta C R & Chatterjea J B, The role of synthetic folic acid ( *L. casei* factor) in the treatment of nutritional macrocytic anaemia, *Indian med Gaz*, **81** (1944) 223.
13. Das Gupta C R & Chatterjea J B, Folic acid in the treatment of macrocytic anaemia in pregnancy, *Indian J med Res*, **37** (1949) 455.
14. Das Gupta C R, Chatterjea J B & Basu P, Vitamin B<sub>12</sub> in nutritional macrocytic anaemia, *Br med J*, **11** (1953) 645.
15. Das Gupta C R, Chatterjea J B & Basu P, Vitamin B<sub>12</sub> in macrocytic anaemia, *Indian med Gaz*, **88** (1953) 1.
16. Das Gupta C R, Chatterjea J B & Basu P, Role of citrovorum factor in nutritional macrocytic anaemia, *Indian J med Ass*, **26** (1956) 1.
17. Das Gupta C R & Chatterjea J B, Relapse in nutritional macrocytic anaemia, *Bull Calc Sch trop Med*, **1**(4) (1954) 7.
18. Chatterjea J B & Das Gupta C R, Dimorphic anaemia, *Indian med Gaz*, **88** (1953) 126.
19. Das Gupta C R, Anaemia in pregnancy—A critical review, *Indian J med Res*, **42** (1954) 411.
20. Napier L E, Das Gupta C R & Mazumder D N, The treatment of hookworm anaemia, *Indian med Gaz*, **76** (1941) 1.
21. Das Gupta C R, The value of transfusion of blood in the treatment of anaemia, *Indian med Gaz*, **82** (1947) 225.

## Darab K Dastur

Dastur, currently Director, Department of Neuropathology and Applied Biology, Bombay Hospital, Bombay, and Honorary Consultant in Neuropathology, Grant Medical College and J J Group of Hospitals, Bombay, has made notable contributions in the field of neurological and neuromuscular disorders, in particular in respect of cerebral blood flow and metabolism in human aging; histopathology and ultrastructure, including nerves in leprosy and in protein-calorie malnutrition; brain and spinal cord in tuberculous meningitis; allergic and viral encephalitides; intracranial and intraspinal tumours, their tissue culture; pathology of muscle disorders; metabolic aspects of human hepatic coma and of Wilson's disease in India; experimental studies on lathyrism, cycasin and isotopic manganese; nutritional disorders of the nervous system; lysosomes in different tissues in disorders of infection and storage; neuropathology of neurotoxins; and fine structure of heart in rheumatic and congenital heart disease.

Dastur plans to continue work in the field of neuropathology with emphasis on (1) study of electronmicroscopic and histochemical changes in some forms of encephalitis, such as rabies, SSPE and slow viruses; (2) continuing observations on rare forms of neurotuberculosis, especially the hypersensitivity reactions of the brain; (3) selected brain tumours,

especially their growth in and fine structure during various stages of tissue culture, and their immunological behaviour; (4) unusual aspects of neuropathies—inflammatory such as leprosy; metabolic, such as diabetic; and in undernutrition, such as PCM; (5) unusual muscle disorders in myopathic, neurogenic and metabolic conditions; (6) CNS in experimental animals in neurotoxic conditions due to anorectic and other compounds; and (7) autonomic nerve fibres and ganglia in the bowel.

Dastur has published about 150 scientific papers, delivered about 50 guest lectures at institutions of neurology, pathology, leprosy or medical science in general, in USA, UK, Austria, Japan, Germany, Australia, India, Israel and certain other countries during 1961-85; and presented about 30 research papers at about 25 international conferences. He was co-editor of one book each on leprosy and neurotuberculosis. He has guided 10 MSc and 10 PhD theses in Applied Biology, Bombay University. He is postgraduate teacher in DM-Neurology, MD-Pathology and MS-Neurosurgery.

Dastur has been associated in various capacities with a number of learned and professional societies, including the Neurological Society, India; Indian Association of Pathologists; Society of



Biological Chemists, India; Association of Physiologists and Pharmacologists, India; Nutrition Society, India; Electronmicroscope Society, India; Research Groups of the World Federation of Neurology on Neuropathology, Neuromuscular Disorders, Tropical Neurology, Neurochemistry and Neurotoxicology.

Dastur is a Fellow of the Indian National Science Academy (1982), Indian Academy of Medical Sciences (1969), and Royal College of Pathologists, London (1976). He was awarded the Basanti Devi Amirchand Prize of the Indian Council of Medical Research in 1982, and DSc of the Bombay University for his then published work in 1971.

### Selected Publications

1. Dastur D K, Cutaneous nerves in leprosy: The relationship between histopathology and cutaneous sensibility, *Brain*, **78** (1955) 615.
2. Dastur D K, The motor unit in leprosy: A clinicopathological study, *Neurology (India)*, **4** (1956) 1.
3. Dastur D K, Antia N H & Divekar S C, The facial nerve in leprosy. II: Pathology, pathogenesis, electromyography and clinical correlations, *Proc 8th Int Cong Neurol*, Vol 11, Vienna, September 1965, 365; *Int J Leprosy*, **34** (1966) 118.
4. Dastur D K, Leprosy—An infectious and immunological disorder of the nervous system, in *Handbook of clinical neurology*, Vol 33, Pt 1, edited by P J Vinken and G W Bruyn (North Holland Publishing Co, Amsterdam) 1978, 421.
5. Dastur D K, Dewan A, Manghani D K & Udani P M, Quantitative histology of nerve in normal children and in children with protein-calorie malnutrition, *Neuropath & appl Neurobiol*, **3** (1977) 405.
6. Dastur D K, Gagrat B M & Manghani D K, Fine structure of muscle in human disuse atrophy, *Abstract 591, Excerpta Medica, International Congress Series No 427, 11th World Congress of Neurology*, Amsterdam, 1977, 198; *Neuropath & appl Neurobiol*, **5** (1978) 85.
7. Dastur D K, Porwal G L, Shah J S & Ravenkar C R, Immunological implications of necrotic, cellular and vascular changes in leprosy neuritis, *Lep Rev*, **53** (1981) 45-65.
8. Dastur D K, Manghani D K, Osuntokun B O, Sourander P & Kondo K, Neuromuscular and related changes in malnutrition—A review, *J neurol Sci*, **55** (1982) 207-30.
9. Dastur D K, Lathyrism, *Wld Neurol*, **3** (1962) 721.
10. Dastur D K, Wadia N H, Desai A D & Sinh G, Medullospinal compression due to atlanto-axial dislocation, and sudden hematomyelia during decompression, *Brain*, **88** (1965) 897.
11. Dastur D K, Prabhakar V & Lalitha V S, Histological analysis of 1000 intracranial space-occupying lesions. I: Age, sex and pattern; and the tuberculomas, *J neurol Sci*, **6** (1968) 575.
12. Dastur D K & Udani P M, Pathology & pathogenesis of tuberculous encephalopathy, *Acta Neuropath*, **6** (1966) 311.
13. Dastur D K & Wadia N H, Spinal meningitides with radiculomyelopathy; II: Pathology and pathogenesis, *J neurol Sci*, **8** (1969) 261.
14. Dastur D K & Lalitha V S, The many facets of neurotuberculosis. An epitome of neuropathology, in *Progress in neuropathology*, Vol II, edited by H M Zimmerman (Grune and Stratton, New York) 1973, 351.
15. Dastur D K & Lalitha V S, Neuroectodermal tumours of the central nervous system. I: Tumours of the neurone series, *Proceedings, National Seminar on Neurooncology*, NIMHANS, Bangalore-560029, August 1979, 165-178.
16. Dastur D K, Fine structure of lysosomes in brain, nerve and muscle, in *Disorders of storage, infection and tissue breakdown*, *Trends Neuro Sci*, **3** (1980) 173.
17. Dastur D K, Lane M H, Hansen D B, Kety S S, Butler R N, Perlin S & Sokoloff L, Effects of aging on cerebral circulation and metabolism in man, in *Human aging—A biological and behavioural study*, edited by J Birren et al., US Department of Health, Education and Welfare, Public Health Service Publication No 986, Washington DC, 1963, 59.
18. Dastur D K, Seshadri R & Talageri V R, Liver brain relationships in hepatic coma with special reference to ammonia and ketoacid metabolism, *AMA Arch Int Med*, **112** (1963) 889.
19. Dastur D K & Manghani D K, Wilson's disease: inherited cuprogenic disorder of liver, brain,

- kidney; in *Scientific Approaches to Clinical Neurology*, edited by Goldensohn and Appel (Lea & Febiger Publishers, New York) 1977, 1033.
20. Dastur D K, Manghani D K & Raghavendran K V, Distribution and fate of Mn 54 in the monkey: Studies of different parts of the central nervous system and other organs, *J clin Invest*, **50** (1971) 9.
21. Dastur D K, Quadros E V, Wadia N H, Desai M M & Bharucha E P, The effect of vegetarianism and smoking on serum vitamin B<sub>12</sub>, thiocyanate and folates in normal subjects, *Br med J*, **3** (1972) 260.
22. Dastur D K, Santhadevi N, Quadros E V, Gagrut B M, Wadia N H, Desai M M, Singhal B S & Bharucha E P, Interrelationships between the B-vitamins in B<sub>12</sub> deficiency neuromyelopathy—A possible malabsorption-malnutrition syndrome, *Am J clin Nutr*, **28** (1975) 1255.
23. Dastur D K, Dewan A, Santhadevi N, Manghani D K & Razzak Z A, Malnutrition—alcoholism, histopathology of nerves, and B-vitamins in nerve, blood and CSF, in *Neurotoxicology*, edited by L Roizin, H Shiraki and N Grcevic (Raven Press, New York) 1977, 529-47.
24. Antia N H & Dastur D K, *Proceedings of Symposium on Leprosy*, University of Bombay and the Vocational Rehabilitation Administration of US Department of Health, Bombay, 1965 (Bombay University Press, Bombay) 1967, 180.
25. Kapila C C, Dastur D K, Singh B & Tandon P N, Tuberculosis of the nervous system, *Proc Symposium, World Health Organisation and Indian Academy of Medical Sciences, Bombay*, 1972 (Indian Academy of Medical Sciences, New Delhi) 1974, 310.
26. Dastur D K & Daver S M, Striated muscle in four categories of leprosy. 2. Fine structural changes. *Int J Lep*, **48** (1980) 149-58.
27. Dastur D K, Vevaina S C, Manghani D K & Shah N A, Changes in atrial biopsies in chronic rheumatic heart disease. I: Cellulo-vascular and mesenchymal reaction, *Path Res Pract*, **179** (1985) 591-99.
28. Dave U P & Dastur D K, Correlation of fine structure with neurotransmitter levels in acute brain oedema in cats, *Indian J med Res*, **81** (1985) 313-24.
29. Dastur D K, Thakkar B K & Desai P R, Experimental neurotoxicity of the anorectic fenfluramine. I: A fine structural model for cerebral lysosomal storage and neuroglial reaction, *Acta Neuropath*, **67** (1985) 142-54.
30. Dastur D K, Review—Cerebral blood flow and metabolism in normal human aging, pathological aging and senile dementia. *J Cerebral Blood Flow Metab*, **5** (1985) 1-9.



## M G Deo

The research activities of Deo have been concerned mainly with three disorders that are prevalent in the tropics: (1) Leprosy, (2) Protein-calorie malnutrition, and (3) Endemic goitre. His work, which has acquired international recognition, is widely cited in highly reputed international text/reference books. Highlights of his research are given below.

### (1) Leprosy

Deo has been working on different facets of leprosy for the last one decade. He has made interesting observations on macrophage functions in different forms of leprosy. His studies on the clinico-pathological spectrum of leprosy, with special reference to histoid leprosy, have been widely appreciated and cited. In 1979, he prepared an 'anti-leprosy' vaccine from ICRC bacilli, which are cultivable mycobacteria isolated from human leproma. During the last 6 years, Phase-I and II clinical trials of the vaccine have been completed.

A vaccine basically acts by enhancing the host immunity. The available laboratory, clinical and epidemiological evidence clearly shows that cell mediated immunity (CMI) is the dominant host immune defect against leprosy germs (*M. leprae*), wherein antibody formation plays an insignificant role. Among the parameters of the CMI, the late lepromin

(Mitsuda) reaction, a type of skin test, exhibits an excellent correlation with the capacity of the host to handle *M. leprae*, in both patients and healthy subjects. An effective anti-leprosy vaccine should, in the first instance, bring about immune (lepromin) conversion in both lepromatous patients and lepromin negative healthy subjects who represent a high risk group, particularly in endemic areas.

The ICRC anti-leprosy vaccine has been tried so far in a number of lepromatous (LL) and borderline patients (BB/BL) and healthy lepromin-negative subjects. The vaccine brings about persistent lepromin conversion in 60% and 95% of LL and BB/BL patients, with upgrading of tissue lesions, associated with tissue bacillary clearance. Some vaccinated LL patients even exhibit reversal reaction. High conversion rates (< 90%) are observed in lepromin-negative healthy subjects who represent a high risk group.

The vaccine is safe and has been found to be non-toxic in different species of animals. In patients too no untoward effects were observed during the 6-year observation period. Given as a single dose, it has high acceptability.

The ICRC vaccine thus meets the requirements of a 'candidate' vaccine which could be used in both

immunoprophylaxis and immunotherapy of leprosy as an adjunct to chemotherapy.

The vaccine has been cleared for Phase-III (mass vaccination) trials, which would begin soon in Maharashtra. This is the first and the only vaccine cleared so far by the Government of India for field trials. Development of a vaccine effective against leprosy is one of the major bio-medical goals of the mankind. Development of ICRC anti-leprosy vaccine would be a landmark in leprosy control.

On the experimental side, Deo has recently shown that Hanuman Langur monkeys (*Presbytis entellus*) exhibit a spectrum of Mitsuda (skin) reaction similar to that observed in man. Further, ICRC anti-leprosy vaccine induces lepromin (immune) conversion in 85% of the negative monkeys. Mitsuda reaction is a good indicator of host immunity against *M. leprae*. The monkey model could be used to screen and identify immunogenic proteins which would be the first step in the development of a synthetic vaccine against leprosy.

## (2) Protein-Calorie Malnutrition

Kwashiorkor, a dominant manifestation of protein-calorie malnutrition (PCM) in young growing children, is a major public health problem in the developing countries, including India. Although clinical and pathological aspects of the disease are fairly well documented, there are many lacunae in our understanding of its pathogenesis.

For every case of PCM, there are large number of cases of sub-clinical forms of the disease. The sub-clinical forms are important not merely because of their large number, but it is at this stage that

malnutrition may contribute towards pathogenesis of several tropical disorders (tropical sprue, nutritional anaemias, osteoporosis). Deo's work, which provides an invaluable source of information, has cleared our notions in these areas.

Besides its applied importance, Deo's work has resulted in new concepts in fundamental aspects of cellular growth and differentiation. He has made in-depth studies on the four fundamental phenomena, namely cell genesis, migration, differentiation and cell death, that are essential for tissue development. His studies have resulted in a new hypothesis of 'number-clock' in the field of chronobiology.

Based on his studies on the cellular aspects, Deo has proposed a concept on cellular reaction to protein deficiency. This concept, briefly stated below, is now widely accepted.

(a) In protein malnutrition, every cell and every tissue is affected, but all are not affected at the same time, nor to the same extent. Deo's observations on sequential alterations and his studies on cell population kinetics have shown that there is a pattern in the reaction of the body to protein depletion. The organs of high protein turnover, such as the liver and pancreas, and those with high rates of cell renewal, such as the intestine and bone marrow, are affected early, whereas the muscle, which has a relatively slow protein turnover, is affected later. He has further shown that at the cellular level, both RNA and DNA are affected. The entire pathological spectrum of PCM can be explained on the basis of these two lesions.



(b) Reduction in cell loss counterbalances diminution in new cell information in organs like the intestine. However, cell differentiation appears to be relatively unaffected. The data suggest that once the number of cells entering the differentiated compartment is curtailed in the germinative zone, no further restrictions are placed on differentiating cells and a smaller compartment of qualitatively normal individual units is produced.

(c) The importance of 'biological clocks' in growth and differentiation of tissues is well documented. The closure of the 'clocks' at a predetermined fixed phase of life has been invoked to explain the irreversibility of lesions induced by malnutrition in early life. Deo's studies indicate that in malnutrition, the closure of the 'clock', particularly in dividing cells, is delayed, permitting them to maintain proliferative activity beyond the stage at which it ceases in the normals. This is an adaptation which enables malnourished organisms to make up, to a considerable extent, the cell deficit, which could result as a consequence of prolongation of the cell cycle.

### (3) Himalayan Endemic Goitre

Deo's work in this field has helped in the formulation of a national goitre control programme through iodization of salt in the endemic areas.

### Selected Publications

1. Deo M G, Bapat C V, Bhalerao V & Chaturvedi R M, Antileprosy potentials of ICRC vaccine—A study in patients and healthy volunteers, *Int J Lepr*, **51** (1983) 540-49.
2. Bhatki W S, Chulawala R G, Bapat C V & Deo M G, Reversal reaction in lepromatous patients induced by a vaccine containing killed ICRC bacilli—A report of five cases, *Int J Lepr*, **51** (1983) 466-72.
3. Deo M G, 'Pro-Eu-Karyote', graft acceptance, a mechanism for intracellular parasitism—A new hypothesis for pathogenesis of leprosy, *Med Hypoth*, **8** (1982) 287-95.
4. Bhutani L K, Bedi J R, Khandhari K C & Deo M G, Histoid leprosy, *Int J Lepr*, **42** (1974) 174.
5. Parmeswaran M, Girdhar B K, Deo M G, Khandhari K C & Bhutani L K, Macrophage function in leprosy, *Int J Lepr*, **44** (1974) 340.
6. Deo M G, Implications of malnutrition in carcinogenesis, *J Oncol Res clin Oncol*, **99** (1981) 77.
7. Deo K, Bijlani V & Deo M G, 'Physiological' and cytotoxic cell death in protein deficiency—A study in developing cerebellum in rats, *Acta Neuropath*, **46** (1970) 221.
8. Deo M G, Cell biology of protein-calorie malnutrition, *Wld Rev Nutr*, **32** (1978) 49.
9. Deo K, Bijlani V & Deo M G, Effects of malnutrition on cell genesis and migration in developing brain, *Exp Neurol*, **62** (1978) 80.
10. Mathur M & Deo M G, Kinetics of proliferation and differentiation in the hair follicle and epidermis in neonatally undernourished rats, *Am J Path*, **82** (1976) 9.
11. Deo M G, Bijlani V & Ramalingaswami V, Nutrition and cellular growth and differentiation, in *Growth and development of the brain*, edited by M A Brazier (Raven Press, New York) 1975, 1-6.
12. Deo M G, Effect of nutrient deficiencies in animals: *Protein CRC Handbook of Nutrition and Food*, Vol II Sect E, 1979, 417.
13. Deo M G & Ramalingaswami V, Regulatory mechanisms of cellular proliferation in protein-deficient organisms, in *Ciba symposium on control processes in multicellular organisms*, edited by G Wholstenholme and J Knight (Churchill, London) 1970, 321.
14. Karmarkar M G, Deo M G, Kochupillai N & Ramalingaswami V, Pathophysiology of endemic goitre, *Am J clin Nutr*, **27** (1974) 96.
15. Sooch S S, Kochupillai N, Deo M G, Karmarkar M G & Ramalingaswami V, Goitre control—Kanga Valley Project, *Bull WHO*, **49** (1973) 307.

## B N Dhawan

My main interest in basic research has been to study modulation of CNS activity by putative neurotransmitters and to analyse the type of receptors involved. The neurotransmitters include the biogenic amines, acetylcholine and enkephalins. The parameters of CNS effect investigated include thermoregulation, central cardiovascular control, analgesia, blood-brain barrier and behavioural disturbances like stereotypy, aggression, etc. The experimental techniques used include microiontophoresis, stereotaxic stimulation, radioligand binding and restricting the drugs to CNS only by appropriate techniques of administration. A summary of the important results is given below.

### (1) Norepinephrine

The presence of alpha-adrenoceptors has been demonstrated in the hypothalamic thermoregulatory loci in several species, including rabbit<sup>1</sup>, pigeon<sup>2</sup> and mastomys<sup>3</sup>. In mastomys, both alpha- and beta-adrenoceptors are involved, the former producing hypothermia and the latter hyperthermia. Adrenoceptors appear to be involved in pyretic responses to several other agents.

In the central cardiovascular loci, alpha-adrenoceptors have been demonstrated to have a predominantly excitatory role employing 6-hydroxydopamine, alpha-

methylnoradrenaline and indoramin as tools<sup>2,4,5</sup>. In addition to changes in blood pressure and heart rate, the effects on optimum and threshold evoked responses from central vasomotor loci have also been studied. The main site of cardiovascular effects of clonidine has been located on the ventral surface of medulla, where an intact cholinergic link is essential<sup>6</sup> for its activity.

The facilitation of flexor reflex in spinal cord is mediated via alpha-adrenoceptors<sup>7</sup>. Beta-adrenoceptors are involved in tremorine-induced tremors<sup>8</sup>. A catecholaminergic link also exists in methylphenidate-induced stereotypy<sup>9</sup> and clonidine-induced aggressive behaviour.

### (2) Dopamine

Involvement of dopamine in the central thermoregulatory mechanism has been demonstrated in rabbit<sup>1,2</sup>, pigeon<sup>2</sup> and mastomys<sup>3</sup>. In the pigeon, the effect is mediated via two types of dopamine receptors<sup>2</sup>, and apomorphine acts via a non-dopaminergic mechanism. Further, a part of dopamine effect in the rabbit and the pigeon is due to its conversion to norepinephrine. In the mastomys, the dopaminergic mechanism is quite distinct from the noradrenergic mechanism and is also involved in amphetamine induced hyperthermia<sup>10</sup>. The role of dopamine in drug-induced stereotyped behaviour has

---

Scientist in Director's Grade, Central Drug Research Institute, Lucknow-226001, Residence : 3 Rama Krishna Marg, Lucknow-226007.



been investigated in pigeon<sup>11</sup> and guineapig<sup>9</sup>. Dopaminergic mechanism is definitely involved in the guineapig, but not in the pigeon. Dopaminergic mechanism is also involved in clonidine-induced aggression. The role of dopamine in the antinociceptive action of morphine was investigated by using agents selectively increasing brain dopamine concentration. Such pretreatment led to a marked potentiation of the analgesic activity of morphine, indicating involvement of a dopaminergic link in morphine analgesia<sup>11</sup>. Microiontophoretic studies on single neurones in prefrontal cortex in rats revealed the presence of specific dopamine sensitive neurones. In addition, on some neurones, norepinephrine and dopamine produced similar effects<sup>2</sup>.

### (3) 5-Hydroxytryptamine (5-HT)

The 5-HT receptors in the central cardiovascular loci were shown to be of undifferentiated type<sup>12</sup>. Reuptake inhibitors of 5-HT have been employed to demonstrate the role of 5-HT in thermoregulation in several species<sup>2</sup>. Activation of serotonergic mechanisms produces hypothermia in pigeons and hyperthermia in mastomys and rabbits. It has been shown that 5-HT is not involved in increasing the permeability of blood-brain barrier<sup>13</sup>.

### (4) Histamine

Both H<sub>1</sub>- and H<sub>2</sub>-type of histamine receptors occur in the central thermoregulatory areas of pigeon<sup>14</sup> and mastomys<sup>2</sup>. In the former species, both of them produce hypothermia, which is dependent on the ambient temperature and is associated with increased heat loss and decreased heat production. In mastomys, hypothermia was obtained only

with smaller doses of histamine and was H<sub>1</sub>-receptor mediated, while hyperthermia was mediated via H<sub>2</sub>-receptors. Histamine is also involved in the regulation of blood-brain barrier, the effect being mediated via H<sub>2</sub>-receptors<sup>15</sup>.

### (5) Acetylcholine

The investigations on cholinergic mechanism have been restricted to medulla: Microiontophoretic application of acetylcholine to neurones of the reticular formation produced both excitatory and inhibitory responses<sup>16</sup>. The inhibitory effects were mimicked by muscarinic agonists and blocked by muscarinic antagonist, whereas the excitatory responses could be antagonized by both muscarinic and nicotinic blocking agents and produced by both groups of agonists. On the ventral surface of medulla<sup>2,6</sup>, microiontophoretically applied clonidine and acetylcholine produce similar effect on 75% of the vasoactive neurones. Further, clonidine can competitively occupy the muscarinic binding sites on neuronal membrane preparation in radioligand binding studies.

### (6) Enkephalins

Topical application of met- and leu-enkephalin and several met-enkephalin analogues to the ventral surface of cat medulla led to a consistent hypotension<sup>17</sup> without significant alteration in the heart rate. Electrically evoked pressor responses were blocked, whereas depressor response to topically applied acetylcholine was potentiated. There was a preponderance of delta receptors in the area, and met-enkephalin may be playing a neuromodulatory role by potentiating cholinergic responses. Opioid receptors are involved in thermoregulation in

mastomys<sup>17</sup>. The predominant effect is hyperthermia mediated via  $\mu$  type of opioid receptors. A cross-tolerance exists between morphine and naturally occurring ligands of opioid receptor in the thermoregulatory loci. The studies have helped in the synthesis of extremely potent met-enkephalin analogues<sup>17</sup>, the most potent being 52,000 times more active.

### Concluding Remarks

These studies, spanning over two decades, have helped in elucidating the role of certain putative neurotransmitters in the regulation of physiological activities by the central nervous system and in identifying the type of receptors involved. These neurotransmitters, however, do not work in isolation. It is, therefore, necessary to study their interactions to get a composite picture. In the projected studies, involving simultaneous use of several neurotransmitters, the increasing number of more specific agonists and antagonists becoming available will be utilized. More sensitive techniques of studying these interactions, like radioligand binding, microiontophoresis, histochemical and scanning electron microscopic techniques, will also be utilized.

### Selected Publications

1. Dhawan B N & Dua P R, Evidence for the presence of alpha-adrenoceptors in the central thermoregulatory mechanism of rabbits, *Br J Pharmacol*, **45** (1971) 497-503.
2. *Current research in pharmacology in India* (1975-1982), edited by P K Das and B N Dhawan (Indian National Science Academy, New Delhi) 1984.
3. Shukla R, Srimal R C & Dhawan B N, The effect of intracerebroventricular administration of catecholamines and their antagonists on rectal temperature of *Mastomys natalensis*, *N S Arch Pharmacol*, **318** (1981) 38-42.
4. Gupta P P, Srimal R C & Dhawan B N, Central cardiovascular effects of 6-hydroxydopamine, *Eur J Pharmacol*, **20** (1972) 215-23.
5. Singh G B, Srimal R C & Dhawan B N, Inhibition by alphamethylnoradrenaline of central vasomotor loci in cat, *Pharm Res Commun*, **5** (1973) 329-36.
6. Dhawan B N, Singh G B & Srimal R C, The effect of clonidine on some centrally evoked cardiovascular responses, in *Recent advances in hypertension*, Vol 1, edited by P Millez and M Safar (Boehringer Ingelheim, Reims, France) 1975, 111-24.
7. Dhawan B N & Sharma J N, Facilitation of flexor reflex in cat by intrathecal catecholamines, *Br J Pharmacol*, **40** (1970) 237-48.
8. Sharma J N, Singh G B & Dhawan B N, Effect of beta-adrenergic blockade on tremorine tremors in mice, *Jap J Pharmacol*, **21** (1971) 675-77.
9. Dhawan B N & Srimal R C, Drug induced stereotyped behaviour in the guineapig, *Neurohumoral correlates of behaviour*, edited by S Subrahmanyam (Thomson Press, New Delhi) 1977, 153-60.
10. Shukla R, Srimal R C & Dhawan B N, Amphetamine induced hyperthermia in *Mastomys natalensis*, in *Homeostasis and thermal stress*, edited G K Cooper, P Lomax, E Schonbaum & W L Veale (Karger, Basel) 1985, 166-69.
11. *Drugs and central synaptic transmission*, edited by P B Bradley and B N Dhawan (Macmillans, London) 1976.
12. Dhawan K N, Dhawan B N & Gupta G P, Nature of 5-HT receptors in central vasomotor loci, *Jap J Pharmacol*, **17** (1967) 435-38.
13. Gulati A, Agarwal S K, Shukla R, Srimal R C & Dhawan B N, Evidence for the lack of serotonergic mechanism in the regulation of blood-brain barrier, *Pharmacol Res Commun*, **16** (1984) 181-88.
14. Shukla R, Srimal R C & Dhawan B N, Analysis of hypothermic response to centrally administered histamine in pigeons, *J Autonomic Pharmacol*, **8** (1983) 373-81.
15. Gulati A, Dhawan K N, Shukla R, Srimal R C & Dhawan B N, Evidence for the involvement of histamine in the regulation of blood-brain barrier



- permeability, *Pharmacol Res Commun*, **17** (1985) 395-404.
16. Bradley P B, Dhawan B N & Wolstencroft J H, Pharmacological properties of cholinceptive neurones in the medulla and pons of the cat, *J Physiol*, **183** (1966) 658-74.
17. *Current status of centrally acting peptides*, edited by B N Dhawan (Pergamon Press, Oxford) 1982.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

## H C Ganguli

Ganguli's major work in psychology has been directed towards developing in depth the areas of industrial and organizational psychology and undertaking researches in the new areas of ergonomics, mental health, interethnic prejudice, international understanding, sexual behaviour and in the field of altered states of consciousness. He has worked in several national institutions of international repute and this undoubtedly gave him a broader outlook and background in psychology than would have been possible if he had worked in a university from the beginning of his career in 1946. These institutions have been: The Employment Selection Bureau, Government of India, New Delhi; All India Institute of Hygiene and Public Health, Calcutta; Indian Institute of Technology, Kharagpur; Indian Institute of Science, Bangalore; Flight Safety Directorate, Indian Air Force; Iraq National Oil Company, Baghdad; and University of Delhi, Delhi. His post-doctoral work in USA under T C M programme, particularly with Dr Paul Fitts at the Laboratory of Aviation Psychology, Ohio State University, during 1957-58, introduced him to ergonomics, an area new to India at that time. He also specialized in student counselling work at the Minnesota University under a USEFI programme.

He established the Department of Psychology at the University of Delhi in 1963 and remained Chairman of this Department for 10 years. Between 1977 and 1980, he established the Post-graduate Department of Psychology at the Tribhuvan University, Nepal on behalf of the Governments of India and Nepal.

Areawise, his first interest was in noise pollution in industries. He did his PhD work in this field, published some papers in *Arbeits-physiologie*, and the *Journal of Institution of Engineers (India)* and authored a book on aircraft noise (Vikas Publications). The latter, together with his paper on control-display relationship published in the *Journal of Aero-Medical Society* and his address at Mussoorie to the Defence R & D groups on the desirability of starting human engineering research in the Armed Forces, did finally find its fruition in the introduction of post-graduate courses in Human Engineering and Aviation Psychology at the University of Delhi.

In industrial psychology, Ganguli's studies made a step-wise progress from incentives and job satisfaction of industrial workers to study of the effects of union membership on workers' attitude and satisfaction (D.Lit. thesis), nature of effective supervision and leadership (Ford



Foundation project), and finally to the study of a complete industrial organization, both in its structural and process aspects (Asia Foundation and IIT, Kharagpur support). As Senior Industrial Psychologist in the Iraq National Oil Company (INOC), Baghdad, he prepared a technical report for an organizational development school in INOC. The last major work in this field was his epidemiological study of prevalence of psychological disorders in an industrial population (ICMR project) and in the air force personnel. Ganguli's presidential address to the Psychology Section of the Indian Science Congress in 1966 was on the theme of mental health in industry. In 1959, he prepared for the WHO Conference on Mental Health and Automation, a working paper; he was also a member of the panel which prepared the WHO Technical Report No. 183 on the same subject. Each of Ganguli's major publications opened up the field for other Indian psychologists. Quantitatively, up to 1972, the Indian Council of Social Sciences Research estimates that 8-9% of all work in the industrial field in India has been authored by Ganguli. His work has also given substance to the area of management psychology; 27 references pertaining to Ganguli's work are given by ICSSR in its survey of research in management in 1973.

From about 1966, Ganguli did several studies in the area of inter-group prejudices in India, particularly with reference to Hindu-Muslim relations. His analysis of Gandhi's fasts and the issues related therein, published in *India Quarterly*, was perhaps the first empirical work in this area. Some of the studies were funded by ICSSR and with data-support from the Home Ministry.

Ganguli's initiation into the area of international understanding occurred as early as in 1966, when he directed a three-country cross-cultural project on images and international understanding with UNESCO funds given to the India International Centre, New Delhi. The interest was continued through his association with UNESCO in its conference on International Understanding and Peace in Paris in 1970 and subsequently through his participation in the International Education Exchange project under Dr Otto Klineberg, funded by the International Social Science Research Council.

In the area of population control, the major effort of Ganguli has been preparation of a paper entitled 'Religion and population explosion' for a Princeton conference, included as a chapter in the book *Religious Pluralism and World Community* (Leiden, 1969). The second major work has been an empirical study on the sexual behaviour of Indians (Ministry of Health & Family Welfare funding). This work is modelled after the Kinsey studies and its objective has been to work out the methodology and standardization of tools. The study was presented at the 1985 Lucknow Science Congress as an invited address for the Prof. Jamuna Prasad Lecture Award of the Indian Psychological Association.

Altered states of consciousness is an intriguing psychological field and Ganguli has empirically studied a Canadian meditation group and published two papers in the *Tavistock Institute Journal of Human Relations*, London. He had extensive discussions on the subject with Maharishi Mahesh Yogi at Seelisberg in Switzerland and with Swami Satyanand

Saraswati at Bihar School of Yoga, Munger. He also lectured on this topic in India as a UGC National Lecturer and in USA at the National Institute of Mental Health and at Cambridge Hospital, Harvard University.

Ganguli is a reviewer of psychological tests for the Buros Mental Measurement Yearbook of the Nebraska University.

Among the awards Ganguli received are: (i) The Delhi Management Association Escorts Award for "outstanding contribution to the understanding of management principles and practices in India" for his book *Structure and Processes of Organisation*; (ii) Sandoz Award of the Indian Psychiatric Society for the best research in mental health by an Indian in 1969, for his work on psychological disorders in Indian industrial population; (iii) Swami Pranavananda East-West National Lecture Award in 1983 for his work on meditation subculture and drug use, (iv) Fourth triennial Prof. Jamuna Prasad Lecture Award of the Indian Psychological Association for his study on sexual behaviour, and (v) Psychology National Award of the Swami Pranavanand Psychology Trust of India for "Outstanding research contribution to psychology in India".

### Future Work

Ganguli's current research interest is in the areas of consciousness, non-violence and peace.

### Selected Publications

1. Ganguli H C, *Industrial productivity and motivation—A psychological analysis* (Asia Publishing House, Bombay) (Reprinted by Allied Publishers) 1983.
2. Ganguli H C, *Structure and processes of organization* (Asia Publishing House, Bombay) (Reprinted by Allied Publishers) 1984.
3. Ganguli H C, *Some thoughts on planning in India* (Asia Publishing House, Bombay) 1967.
4. Ganguli H C & Prakash Rao M S, *Human factors aspects of aircrafts noise* (Vikas Publication, New Delhi) 1971.
5. Ganguli H C, *Foreign students: The Indian experience* (Sterling, New Delhi) 1975.
6. Ganguli H C, Religion and population explosion: Numen supplement, XV, in *Religious pluralism and world community; Interfaith and intercultural communication*, edited by Edward J Jurji (EJ Brill, Leiden) 1969.
7. Ganguli H C, International education exchange—National study in India, in *International educational exchange*, edited by Otto Klineberg (Mouton, The Hague) 1976.
8. Ganguli H C, Mehrotra G P & Howard D Mehlinger, Values, moral education and social studies, in *UNESCO handbook for the teaching of social studies* (Croom Helm, London/ UNESCO, Paris) 1981.
9. Ganguli H C, *Mental health problem of automated and non-automated workers with reference to accelerated industrialization*, Paper presented to the World Health Organisation Study Group on Automation and Mental Health Problems, Geneva, 10-15 November 1958.
10. Ganguli H C, *A social-psychological analysis of international understanding and conflict*, Paper published by UNESCO for limited distribution for Meeting of Experts on Education for International Undertaking and Peace with Special Reference to Moral and Civic Education; No ED/Conf 23/10, Paris, 17-28 August 1970.
11. Ganguli H C, *Images and international understanding: UNESCO Project Report: Parts I and II* (Research Council for Cultural Studies, India International Centre, New Delhi) 1966-67.
12. Ganguli H C, *Report on An Organization Development School for Iraq National Oil Company* (Iraq National Oil Company, Baghdad) October 1974.
13. Ganguli H C & Rao M N, Noise and industrial efficiency: A study of Indian jute weavers, *Arbeitsphysiologie*, **15**(5) (1954) 344-54.
14. Ganguli H C, Attitudes of union and non-union employees in a Calcutta electrical engineering factory, *Am J appl Psychol*, **40** (1956) 78-82.
15. Ganguli H C, Isolation of some morale dimensions by factory analysis, *Sankhya*, **17**(4) (1957) 393-400.



16. Ganguli H C, Stimulus-response compatibility and stress effects in a one-dimensional tracking task, *Aero Med Soc J* (July 1960) 11-16.
17. Ganguli H C, Human engineering research and the defence services, Paper presented before the Mussoorie Conference on Military Psychology, June 1960, *Def Sci J*, **11**(3) (1961) 193-210.
18. Ganguli H C, Psychological disorders in air force personnel, *Def Sci J*, **3A**(2) (1963) 1-14.
19. Ganguli H C, Prevalence of psychological disorders in an Indian industrial population: Parts I, II and III, *Indian J med Res*, **56** (1968) 754-60.
20. Ganguli H C Psychological Research in India: 1920-1967, *Int J Psychol*, **2** (1971) 165-77.
21. Ganguli H C, Hindu-Muslim problem in the Gandhian programme, *India Quart, New Delhi*, (1970) 390-409.
22. Ganguli H C, Dynamics of international conflict, *India Quart, New Delhi* (July-September 1972) 1-29.
23. Ganguli H C, Prejudice and its social consequences, *Indian J Soc Work, Bombay*, **33** (1972) 243-56.
24. Ganguli H C, Rajni Jain & Rita Jalali, An interactional study of superior-subordinate relationship, *Indian J ind Relat*, **4** (1983) 499-516.
25. Ganguli H C, Meditation programme and modern youth: Dynamics of initiation, *Human Relations, London*, **35**(10) (1982) 903-26.
26. Ganguli H C, Meditation subculture and drug use, *Human Relations, Lond*, **38**(10) (1985) 953-62.
27. Ganguli H C, Study of sexual behaviour, *Personality Study & Group Behaviour*, **V**(1) (1985) 25-67.
28. Ganguli H C, Non-violence as an attitude: Behaviour principle and modern life; Social change, *J Coun Soc Devel*, **15**(1) (1985) 3-12.

# T K Ghosh

## **(A) Cardiovascular Work (1940-45)**

During this period, as a research scholar and research associate under Dr Bidhan Chandra Roy, Director, Sir Nilratan Sircar Research Institute, Carmichael Medical College (R G Kar Medical College), Calcutta, Ghosh developed a methodology of research in clinical medicine as yet unavailable in the country. The methodology was evolved as a model involving the use of instrumentation for venous pressure estimation, oscillotometry, plethysmography, skin temperature, ECG and other investigative procedures to study cardiovascular disorders.

Arterioral dilatation in epidemic dropsy as a diagnostic aid and clarification of pathogenesis in this serious disease, marked increase in oscillomycine index in essential hypertension as distinct from hypertension caused by renal and other causes, arterioral dilatation in normal sleep, vasoconstriction in congestive cardiac failure as a compensatory phenomenon with arterioral dilatations at the onset of sleep leading to hyperovolemia cardiac dyspnoea, arterioral dilatation in aortic insufficiency and anemia were among the original contributions included in the doctoral thesis<sup>1</sup>.

## **(B) Neurology Work (1945-70)**

In the field of neurology, the following original contributions were made: A new concept of function of frontal lobe in its projective function for future<sup>2</sup>, clinically useful and reliable psychometric tests of "picture drawing" to distinguish mental symptoms due to psychitric disorder from organic brain diseases based on follow-up of patients with verified brain tumour<sup>3</sup>, autonomic dysfunction in frontal lobe disorders<sup>4</sup>, increased volume pulsation in paralysed limbs in hemiplegia with subsequent reduction of volume pulsation on recovery<sup>5</sup>, autonomic dysfunction in cerebral diplegia<sup>5</sup>, and diagnostic assessment of electromyographic changes in 1000 cases of neuromuscular disorders<sup>7</sup>.

## **(C) Electroencephelographic Work (1951-1980)**

The notable achievements in this field include: Realization of the significance of spike and wave complexes and diagnostic value of EEG for the first time in India<sup>8</sup>; EEG changes in recurring episode; prolonged disorders of behaviour for 4-10 days noted as distinct from epilepsy (of transient duration) and schizophrenia (of longer duration), a significant borderline syndrome reported for the first time<sup>9</sup>, and observations on 22,253 EEG records, including 17,075 epilepsy and 625 brain tumour cases<sup>10</sup>.



**(D) Epilepsy Work (1949-1985)**

*Clinical* : Since 1949, careful records on special case sheets of significant features, including investigative procedures (EEG, etc.) and drug therapy of patients with epilepsy, were kept and followed up systematically. A sizeable data on different facets of epilepsy have accumulated, including long-term follow-up over 6-21 years and carefully conducted in-depth study of short-term follow-up over 4-10 years.

*Long-term follow-up* : A long-term follow-up of 17,075 epileptic patients was done for a period of 21 years during 1951-71. Epileptic patients numbering 10,423, who had their initial registration during 1952-56, were selected for final follow-up during 1972-73 to provide a minimum of 6 years and maximum of 21 years of follow-up.

Six hundred random samples were drawn from 10,075 patients and data on initial (pre-treatment) and final (post-treatment) attendance of 600 random samples were recorded in specially prepared case sheets and subsequently transferred to code sheets and punch cards; the information thus obtained was subjected to electronic data processing, yielding 241 tables which were statistically analysed including  $X^2$  tests.

*Short-term close follow-up* : Five hundred patients beginning with a random number were selected for in-depth and close follow-up in special case sheets and weekly/fortnightly/monthly follow-up was done from 1969-70 to 1975-76 to give 6 years follow-up, yielding 17,20,000 bits of information which were subjected to electronic data processing to give 723

tables, which were statistically analysed including  $X^2$  tests.

Two hundred and ninety patients from a group of 500 of 1969-70 were also followed up fortnightly/monthly till 1980 to give information of prospective follow-up of 10 years to give 420 bits of information in 27 tables, specially in relation to how long should anticonvulsant drugs be continued after control of seizure.

Reports of similar studies in epilepsy—long-term follow-up over 21 years and close in-depth, short-term study of 10 years on clinical, psychological, socio-economic, medication aspects—are not available in medical literature, national or international, and as such could not be compared with others. A few interesting features observed from this large reservoir of information on epilepsy which is being published as a monograph are as follows :

Incidence of epilepsy is commoner in males than in females (3:1). Patients were brought for initial examination at an average age of 17 years (lowest 4 months and highest 63 years). The average age of onset of epilepsy was 15 years for males and 13 years for females (shortest 3 days and longest 56 years). Untreated period was on the average 3 years (minimum 1 day and maximum 31 years). Post-ictal phenomenon was present in 83% cases. The frequency of seizure was 1-9 per year in 47.3% and 100 or more in 21-50% cases. The intensity of seizure was severe in 12% cases. Family history of epilepsy was present in 18% cases. Genetic predisposition was noted in 22.50% cases. About 40% had complete control of seizures. During follow-up, abnormality in personality showed an increase in 9%, of emotional status in 16%, intellectual deterioration in 11%, and unemployment in

11% cases. Marriageability was reduced by 42% in females and in 5% males after epilepsy. No epileptic father got any epileptic child, but epileptic mothers had epileptic children. Children below 5 years of age suffering from epilepsy showed increased emotional and personality changes and deterioration in intellectual and educational status. Irregularity in intake of medicine produced recurrence of seizures. Early use of drugs continued up to 4 years gave better results.

### (E) Neuro-chemical Studies

Experimental neuro-chemical studies on the action of pentamethylenetetrazol on brain serotonin of rabbits showed that a single dose of pentamethylenetetrazol (PMT) administered i.v. to male rabbits, weighing 1200-1600 kg both at low (25 mg/kg) and high (40 mg/kg) doses, increases the serotonin (5-HT) content of whole brain to a maximum amount at 60 min. Higher drug dose produces a more pronounced effect. Measurement of turnover rates (non-isotopically) of brain 5-HT showed that PMT, at both low and high doses, inhibits the synthesis rate as well as 5-hydroxyindoleacetic acid (5-IAA) accumulation rate and declination rate. The monoamine oxidase (MAO) activity is inhibited by PMT under similar conditions of treatment. The possible mechanism of PMT-induced convulsion at the level of brain 5-HT was discussed<sup>11</sup>.

The action of pentamethylenetetrazol on (a) brain-synaptosomal ACHE, (b) mitochondrial MAO activity, and (c) brain serotonin metabolism was also studied.

In clinical correlative studies, MAO activity on blood platelets of epileptic patients—age and sex matched in different

types of epilepsy with time lapse, before and after a seizure—was measured to assess whether it will be helpful in predicting the onset of a seizure.

### Selected Publications

1. Ghosh T K, *Clinical studies on oscillotonometry in health & diseases*, PhD thesis, University of Calcutta, 1941.
2. Ghosh T K, Function of frontal lobe—Concepts changing, *Annual Volume, Physiology and Experimental Medical Sciences, Calcutta* (Sherrington Anniversary Volume) 1958, 115.
3. Ghosh T K & Chandra N, A psychometric study of some aspects of ability in the case of a right frontoparietal tumour before and after operation, *Bull Inst Post-grad med Educ Res, Calcutta*, 4 (1962) 58.
4. Ghosh T K, Studies-correlative-on psychosomatic and autonomic dysfunction in verified role lesion, *Final Report, ICMR Project*, 1962.
5. Ghosh T K & Mukherjee J, Plethysmographic studies in hemiplegia, *Bull Inst Postgrad med Educ Res, Calcutta*, 5 (1963) 50.
6. Ghosh T K & Ghosh T C, Skin temperature in normal individuals and in case of cerebral diplegia, *Bull Inst Postgrad med Educ Res, Calcutta*, 1 (1959) 121.
7. Ghosh T K, Electromyographic study of 1000 neuro-muscular disorders with comment as to its significance, *Symposium on Electromyography in Neuromuscular Disorder*, Bombay, 1969, 35.
8. Ghosh T K, Studies on epilepsy—Diagnostic value of EEG, *Trans Annu Conf Neurolog Soc, Hyderabad*, 1951, 14.
9. Ghosh T K, Electroencephalographic changes in recurring episodes of prolonged disorder of behaviour, *Bull Univ Coll Med, Calcutta*. 1 (1963) 44.
10. Ghosh T K, Convulsive seizure—A long-term follow-up study, *Seminar on Epilepsy*, WHO and Indian Academy of Medical Sciences, Bangalore, 1975.
11. Ghosh T K, Poddar M K & Ghosh J J, Action of pentamethylene tetrazol on brain serotonin, *Domgs exp Clin Res*, 3(2) (1977) 35-38.
12. Ghosh T K, Clinical significance of estimation of MAO activity on blood platelets or epileptic patients, *J Indian med Ass*, submitted for publication.



## C Gopalan

Gopalan's research contributions relate to the currently widespread problems of human nutrition among under-privileged communities. These include over 200 papers in journals in India and abroad, and contributions to over a dozen books on nutrition. His major contributions are summarized below.

### **(1) Studies on Protein-Calorie-Malnutrition (PCM)**

PCM and its more spectacular clinical manifestations, kwashiorkor and marasmus, are now recognized as perhaps the most pervasive problems afflicting children in India and many other developing countries. Gopalan published numerous papers based on his researches on this subject. In one of these<sup>1</sup>, he presented the first convincing demonstration that the basic deficiency underlying kwashiorkor, marasmus and PCM (in general) was calorie deficiency and not protein deficiency as was till then generally believed, and that there was no basic difference in this regard as to the dietary background between kwashiorkor and marasmus. Though this view was strongly opposed at the time it was advanced, it is now widely accepted and has served to change the concept and strategy with regard to the prevention and control of this major nutrition problem of children. Further evidence in support of

the above view was provided in two papers<sup>2,3</sup>.

Results of studies on the biochemical and functional effects of PCM and on its short-term and long-term implications with respect to growth, development, mental function, immunological status and future physical performance were presented by Gopalan along with his colleagues in over 25 publications in Indian and foreign journals.

### **(2) Studies on Famine Oedema**

Studies on the effects of chronic starvation have provided insights into the clinical and biochemical effects of chronic famine<sup>4-7</sup>.

Results of other studies on basal metabolism, effects of nutritional rehabilitation, electrolyte changes, electrocardiographic changes and hormone status were presented in 7 joint publications with colleagues.

### **(3) Studies on Human Milk and Lactation**

Studies in this area have provided important data on the output and chemical composition of human milk of Indian mothers of poor socio-economic groups, and as a basis for action programmes designed to promote breast feeding, which continues to be the sheet-anchor of infant nutrition<sup>8-10</sup>.

---

Formerly, Director-General, Indian Council of Medical Research, Ansari Nagar, New Delhi-110029;  
Residence : c/o The Nutrition Foundation of India, B-37, Gulmohar Park, New Delhi-110049.

These studies revealed for the first time that despite their poor nutritional status, Indian women of the poorest communities secreted as much as 400-600 ml milk daily for periods extending to well over 12 months; the protein concentrate of the milk was surprisingly good, though not the vitamin concentration. The latter, as well as the total output, could be raised through provision of nutritional supplementation to the mother.

The results of several other studies on the chemical composition of human milk, effect of nutritional supplementation on chemical composition and nitrogen balance in women during lactation have been published jointly with colleagues in six other publications.

#### (4) Studies on Pellagra

The series of papers in this area have served to challenge the current concept that pellagra was a disease of maize-eaters and to advance the view that apart from tryptophan deficiency, amino acid imbalance arising from excess leucine in the diet could be causative. One paper<sup>11</sup> summarizes the several publications of Gopalan and his colleagues on the role of leucine in the pathogenesis of pellagra and underlines the fact that pellagra is by no means confined to maize-eaters only, but also afflicts populations subsisting on millets which are not deficient in tryptophan.

#### (5) Studies on Aflatoxins

The problem of fungal contamination of groundnuts and other foods is now recognized as of serious public health significance. One of the papers<sup>12</sup> was the first demonstration that aflatoxin could produce hepatic carcinoma in primates.

#### (6) Other Studies

Notable among Gopalan's several other publications relate to nutrition and his thirteenth Jawaharlal Nehru Memorial lecture 'The child in India'<sup>13</sup> and his paper<sup>14</sup> challenging the current "classifications" of undernutrition purely on the basis of growth retardation.

#### Overall Contribution

As Director, National Institute of Nutrition for nearly 15 years, Director-General, ICMR for over 5 years and as President of the Nutrition Foundation of India since 1980, Gopalan, apart from his individual contributions, has helped promote research into problems of human nutrition. He founded the Nutrition Society of India, was the first President of the Indian Dietetic Association, President of the First Asian Congress of Nutrition, First Chairman of the Regional Advisory Committee of Medical Research of WHO, Chairman of the Technical Session of World Health Assembly in 1977, and President, International Union of Nutrition Sciences (affiliated to ICSW) from 1975 to 1979.

#### Selected Publications

1. Gopalan C, Kwashiorkor and marasmus—Evolution and distinguishing features, *Proc Colloquium on Protein Deficiency*, edited by R A McCance and E M Widdowson (Churchill, London) 1967, 49-58.
2. Gopalan C, Nutritional constraints on growth and development in current Indian dietaries, *Indian J med Res*, **59** (1971) 111-22.
3. Gopalan C, Swaminathan M C, Krishnakumar V K, Hanumantha Rao D & Vijayaraghavan K, Effect of calorie supplementation on growth of undernourished children, *Am J clin Nutr*, **26** (1973) 536-66.
4. Gopalan C, Anti-diuretic factor in the urines of patients with nutritional oedema, *Lancet* (i) (1950), 304-6.



5. Gopalan C, Venkatachalam P S, Someswara Rao K & Menon P S, *Indian J med Sci*, **6** (1952) 277-95.
6. Gopalan C, Venkatachalam P S & Srikantia S G, Body composition in nutritional oedema, *Metabolism*, **2** (1953) 333-43.
7. Gopalan C, Famine oedema, in *International encyclopaedia on food and nutrition*, edited by H M Sinclair (Pergamon Press) 1969, 19.
8. Gopalan C, Studies in lactation in poor Indian communities, *J trop Paed*, **4** (1957) 87-97.
9. Gopalan C, Protein-intake of breast-fed poor Indian infants, *J trop Paed*, **2** (1956) 85-92.
10. Gopalan C, Nutrition and lactation, *Fed Proc*, **20** (1961) 177-84.
11. Gopalan C, Pellagra and amino-acid imbalance, in *Vitamins and hormones*, Vol 33 (Academic Press) 1975, 505-28.
12. Madhavan T V & Gopalan C, Effect of dietary protein on carcinogenesis of aflatoxin, *Arch Path*, **85** (1968) 133-37.
13. Gopalan C, The child in India, *Thirteenth Jawaharlal Nehru Memorial Lecture*, Jawaharlal Nehru Memorial Fund, New Delhi, 1980, 125-51.
14. Gopalan C & Jaya Rao Kamala S, Classification of undernutrition—Their limitations and fallacies, *J trop Paed*, **30** (1984) 7-10.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

## N Gopinath

From morphologic and functional studies on human aortic and mitral valves, besides the well known anatomical information, it was found that the tensile strength of aortic wall varied between 650 and 1500 g, the average value being 1100 g. The cuspal strength was relatively less than that of the aortic wall. The average cuspal strength was 700 g. From a detailed study on 50 human aortic valves, good correlation was found between tensile strengths of the aortic wall and the valve cusps.

Autopsy study of 20 human mitral valves in Indians revealed the average circumference of the valve in males to be 9.1 cm and in females 8.3 cm; these values are less than those for western people. The commissural and papillary muscle length and width of cusps were otherwise equal to those reported for western populations. Posterior cusps had 3-5 scallops. The function of the mitral valves was not altered if one of the scallops of the posterior cusp near the commissure was compromised in plastic repair of the mitral valve.

From a study on the biochemical and biophysical properties of 20 heart valves, the collagen content of heart valves was found to be approximately 9% of its wet weight, much higher than in other cardiovascular tissues. A study involving enzymatic treatment with pepsin, trypsin

pepsin digestion, collagen estimation based on hydroxy protein content was done. Mucopolysaccharide was evaluated by measuring sialic acid and hexose. Study of 10 normal and 10 rheumatic human aortic and mitral valves showed 10% increased protein level in rheumatic valve, reflecting fibroblastic proliferation and collagen formation. There is increase in deposition of mucopolysaccharide to a similar extent. Electronmicroscopic study of human aortic valves showed collagen fibres in dense parallel bundles and in crimped form. Elastin fibres formed a network, holding the collagen fibres along them and also across. The formation is crucial to the function of valve in the aortic diastolic phase, enabling it to take the strain. A similar study on the mitral valves showed an increased layer of spongiosa between atrialis and fibrosa.

Collagen was present in greater strength on the ventricular aspect. In rheumatic process, the changes seen in mitral valve are separation of collagen fibres, disruption of elastic fibres, loss of architecture with entrapping of platelets and disrupted blood cells with associated increase in collagen and even calcification.

Rheumatic infection of heart can affect very young people. Study of 126 patients under 20 years of age (average 15.5 years) with rheumatic mitral valve disease was



reported as early as in 1964. They formed 34% of rheumatic patients. Carditis was evident within 5 years of rheumatic infection in 83 of them. The degree of incapacity, low incidence of embolism (1%) and auricular fibrillation (2%) and calcification (5%) of mitral valve were the highlights. There was severe pulmonary hypertension with direct left atrial pressure measuring over 23 mm Hg in series of 22 patients. There was no correlation between histological evidence of reactivity from atrial biopsy and rheumatic recurrence. During the follow-up period of 1-7 years, 15% of patients had rheumatic activity. Mortality was low, with 96% cases showing fair to good results during the follow-up period.

Immunologic studies were carried out on rheumatic heart valves. Precise identification of the phenolyptic profile of inflammatory mononuclear cells was attempted. Besides mononuclear cells, the lymphoid infiltrate had predominance of T cells (70-80%) and only occasional B cells. Most of the T cells were OKT 4 positive with only minor representation of suppressor/cytotoxic OKT 8 positive T cells. OKT 4 positive T cell collections were closely juxtaposed to fibroblasts and collagen fibrils. The study suggests that chronic rheumatic scarring process may involve helper/inducer T cells as an ancillary factor in slow fibrosis of deformed cardiac valves. Attempts to show residual streptococcal antigen by indirect immunofluorescence having wide panel of heterologous rabbit F (ab')<sub>2</sub> reagents with specificity for group A streptococcal hydrate gave negative results. This suggests genetic influence in immune response of the host on repeated episodes of Group A streptococcal pharyngitis. This may represent cell mediated immunity

generated by memory T cells. These T cells may be originally sensitized to streptococcal antigens which produce inflammatory response against cardiac antigens altered by the rheumatic process. Thus, they may share antigenic sites with streptococcal material.

Unstable angina is a life threatening syndrome of coronary artery disease. Analysis of 72 patients with this lesion showed normal coronary arteriogram in 9. The left main coronary artery may be involved in a good number of cases (15); majority of the patients were under 50 years of age.

Myocardial dysfunction in constructive pericarditis has been found to cause incomplete relief after surgery. Improvement in function was proved by hemodynamic studies after acute digitalization. In 33 patients, 69 hemodynamic studies were conducted. In 20 patients after acute digitalization, studies were repeated with increase in cardiac output and lower filling pressures. Routine digitalization was indicated in management, especially after surgery.

Noncirrhotic portal fibrosis forms one quarter of cases of portal hypertension in India (75 out of 300 cases). Marked splenomegaly with recurrent haemorrhage from oesophageal with minimal hepatocellular failure are clinical characteristics, suggesting a separate clinical entity. Hemodynamically, there is a marked gradient between splenic pressure and wedged hepatic vein pressure and normal hepatic blood flow. Radiologically, there was not much distortion of hepatic vascular pattern in contrast to cirrhosis. Liver biopsy examination showed focal occlusive changes in medium sized radicals of the portal vein, widening of and

collagenization of space of Disse with large collagen bundles in and around the sinusoids. The group has to be separated from cirrhosis of liver and surgical results are better, with 10% mortality.

Endoscopic, radiological and pathological studies in pulmonary tuberculosis showed infiltration of bronchi segmental and lobar in 30% of cases. Bronchial stenosis worsening with treatment due to fibrosis occurs in 25% of the cases. This is an important finding to be considered in management, especially for cases showing poor response.

### Selected Publications

1. Khanna S K, Gopinath N, Talwar J R & Chandra J, Morphological and functional study of human aortic valves, *Indian J Surg*, **38** (1976) 485.
2. Khanna S K, Talwar J R, Gopinath N & Chandra J, Morphological valve, *Indian Heart J*, **28** (1975) 45.
3. George Cherian, Vytilingam K I, Sukumar I P & Gopinath N, Mitral valvotomy in young patients, *Br Heart J*, **26** (1964) 157.
4. Iyer K S, Gupta A, Kaul U, Balaram A, Punnose U A, Rajani M, Bhatia M L, Sampat Kumar A, Rao I M, Venugopal P & Gopinath N, Prosthetic valve replacement under 20 years of age, *Indian Heart J*, **34** (1982) 314.
5. Iyer K S, Reddy K S, Rao I M, Venugopal P, Bhatia M L & Gopinath N, Valve replacement in children under twenty years of age, experience with Bjorck-Shiley prosthesis, *J Thoracic Cardiovasc Surg*, **88** (1984) 217.
6. Raizada U, Williams J C (Jr), Chopra P, Gopinath N, Prakash K, Sharma K B, Cherian K M, Arora R, Nigam M & Zabriskie J B, Tissue distribution of lymphocytes in rheumatic heart valves as defined by monoclonal T cell antibodies, *Am J Med*, **74** (1983) 90.
7. Gopinath N, Clinical profile and surgical experience of young patients with coronary disease, *India Heart J*, **36** (1984) 286.
8. Gupta A, Iyer K S, Choudhury D, Balaram A, Reddy K S, Bhatia M L, Punnose U A, Sampath Kumar A & Gopinath N, Chronic constructive pericarditis—A surgical experience, *Indian Heart J*, **34** (1982) 332.
9. Sama S K, Bhargava S, Gopinath N, Talwar J R, Nayak N C & Tandon B N, Noncirrhotic portal fibrosis, *Am J Med*, **51** (1971) 160.
10. Betts R H, Thomas T & Gopinath N, Changing concepts in the indications of pulmonary resection, *Indian J Tuberc*, **11** (1955) 41.



## Robert Heilig

On return from the battlefield as a lieutenant in horse artillery, in 1918, I started medical clinical work, while still a student, at Prof. K F Wenckebach's Medical University Clinic in Vienna in 1919 and soon discovered in cooperation with P Saxl the diuretic effect of Novasurol "Bayer"<sup>1</sup>, an injectable mercury preparation, used till then exclusively as an antisyphilitic drug. We used it in cases of cardiac oedema only, strictly excluding renal oedema, because of its high mercury content. The diuretic effect proved exceedingly strong, the loss of water and chlorides following a Novasurol injection being very high; within a year or two, Salyrgan was introduced. It showed a similar effect as Novasurol, but the adverse effect was largely mitigated, so that it replaced our discovery. Still, Novasurol remains as the first injectable mercurial diuretic to be introduced.

Nothing was known about the effects of menstruation on metabolism. Investigations on its influence on blood sugar, water-salt metabolism, liver function, composition of the cerebro-spinal fluid, and skin reactions were undertaken<sup>2,3</sup>.

Entirely different investigations were carried out almost simultaneously, with the neuropsychiatrist Hans Hoff, on the effect of suggestions or orders given to hypnotized persons on gastric function, on

renal function<sup>4</sup> and on the development of herpes labialis; the result was proof beyond doubt that psychosomatic connections regulate these organ functions. Under the suggestion, given to normal hypnotized subjects (my wife, nurses, etc), that food is being eaten that the individual normally dislikes, such as, for instance, onion in the case of my wife, gastric acid secretion ceases completely, the stomach, examined radiologically, loses its tonus, hanging down like a bag and, under continued suggestion of such undesired food intake, retrograde peristalsis leads to nausea and attempts to vomit. Similarly dependent on suggested well-being or its contrary under hypnosis proved renal function, where unhappiness caused increased loss of water and chloride. Finally, a young man, who complained of herpetic eruptions following stress situations, when put under hypnosis and suggested that he was exposed to such stress, for instance, playing football against too strong adversaries, developed labial herpes in the same way as after such an experience in real life.

After my shifting to India, caused by German invasion of Austria, my research work was devoted to malaria, hookworm disease typhus and, finally, to population control.

Studies on malarial nephritis<sup>5</sup> and fluid retention in ankylostomiasis<sup>6,7</sup> were carried out simultaneously with the discovery of typhus cases in Mysore, not reported before. A series of hookworm investigations followed, dealing with the influence of anti-anaemic treatment on gastric function and particularly on the heart condition as seen in serial electrocardiograms, and orthodiagrams. They went from severe myocardial depression and cardiac enlargement at the height of anaemia to normal when iron therapy relieved the anaemia<sup>8,9</sup>.

Typhus was diagnosed first in a schoolboy; this diagnosis was strongly opposed by local colleagues when the case was first demonstrated; these doubts seemed confirmed when the first Weil-Felix tests, specific for typhus, were negative; but soon V R Naidu, our pathologist, grasped the secrets of the agglutination test, found it positive in all the following cases, equally diagnosed as typhus. From the first, the details of our agglutination results were different from those for all the other typhus groups known till then. At the request of N H Topping, I sent three of our typhus cases to his institute at Bethesda, Maryland, then the world centre of typhus research. He fully confirmed our results and since then our type was generally recognized as "Mysore thyphus".

During my tenure as Chief Physician and, from the start of S M S Medical College, Jaipur, as Professor of Medicine there, I started preaching population control; eight of my publications on this subject, imploring the authorities to induce people to practise this most essential of all controls, appeared during 1965-1971.

Many other publications, 180 in all, appeared dealing mainly with prevention of cardiac incidents, especially coronary insufficiency. Papers on amoebiasis, infective hepatitis, vitamin deficiency, and various medico-historical subjects, including the history of Indian medicine, make up a fairly varied list of publications.

As far as planning for future work (as demanded by the editors of this volume) is concerned, at the age of 88, I prefer leaving it to a future incarnation.

### Selected Publications

1. Sexl P & Heilig R, *Wiener Klin Wo*, **33** (1920) 943.
2. Heilig R, *Klin Wo*, **3** (1924) 1117.
3. Heilig R & Kantiengar N L, *Ann int Med*, **16** (1942) 538.
4. Heilig R, *Indian med Gaz*, **76** (1941) 519.
5. Heilig R & Naidu V R, *Indian med Gaz*, **76** (1941) 705; **77** (1942) 338; **79** (1944) 154.
6. Troping N H, Heilig R & Naidu V R, *Publ Hlth Rep*, **58** (1943) 1208.
7. Heilig R, *Indian med Gaz*, **77** (1942) 257.
8. Heilig R & Visveswar, *Indian med Gaz*, **77** (1942) 395; *Indian med Gaz*, **78** (1943) 578.



## K R Laumas

A brief account of the major research contributions of Laumas is given below.

### **(1) Development of Newer Long-acting Hormonal Contraceptives**

The contraceptive steroid which has to be taken daily is highly effective in fertility control; however, failure to take the pill daily results in pregnancies. There is need to develop methods which are safe, effective and long-acting and do not require constant follow-up. Laumas has made numerous contributions to the development of new delivery systems for long-acting contraceptive methods.

Significant work has been done by Laumas on the development of a single silastic implant method for long-term contraception in women. The initial work of Laumas and his students and colleagues revealed norethidrone acetate to have a high degree of permeability through silastic tubing. This could provide a single implant with sufficiently high release to give long-term contraception in women. Animal experiments confirmed the effectiveness of the single implant. Initial trials in women indicated that the use of a single silastic implant provided an excellent approach to long-term contraception, which could be reversed at any time by the removal of the implant. Subsequently, considerable amount of work has been carried out to

ensure the safety and effectiveness of the method, study of the effect of the steroid released from the implant on bleeding problems and other side effects in women. Pharmacokinetic studies with norethidrone acetate have been carried out. The mechanism of action has been evaluated through the study of the levels of circulating hormone in women using the implant. Endometrial histology has been correlated with the circulating levels of the steroid. Gas chromatographic and mass spectrometric methods and radioimmunoassays have been developed to measure the quantity of the steroid released from the implant. A three-centre clinical trial in Delhi and Bombay established the effectiveness of the method. The clinical trials included women from both rural and urban populations. The data revealed a high degree of acceptability of the single implant method approach to long-term contraception among rural and urban populations. Laumas, through his overwhelming contributions to the development of the single silastic implant for contraception, has contributed immensely to the field of human reproduction and fertility control.

His other contributions relate to the development of a vaginal ring for female contraception. He introduced a new innovation in the development of this

---

Formerly, Professor & Head, Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi; Residence : 88-44 247 Street, Bellerose, NY 11426, USA.

device. The vaginal ring could be used from day 5 to 25 of the menstrual cycle of the women.

## **(2) Mechanism of Action of Steroid Hormones**

For the first time it was demonstrated that progesterone is rapidly taken up by the uterus; initially it disappears rapidly and is then retained in the uterus. The next common goal of the work on the mode of action of hormones was to understand the mechanism of action of progesterone and synthetic progestins in the human uterine, endometrial and myometrial tissues. The contributions to this field are unique in many ways. The initial studies demonstrated high affinity and specific binding of progestins, specifically norethindrone, with human uterine cytoplasmic receptors. Laumas and colleagues then isolated and purified the uterine receptor using sequentially density gradient, ion-exchange chromatography and isoelectric focusing techniques. By using gradient displacement and competitive radioassays, a number of estrogenic and progestational compounds, both agonists and antagonists, were tested for evaluating structural requirement for efficient binding to the uterine receptor. The most conspicuous common feature of the 3-oxo-4-ene structure for all the high affinity binders thus became apparent.

Another significant study in this area has been on the down regulation of the uterine receptors for estrogens and progestins. Laumas and colleagues found that both these receptors were under cyclic regulation in the human uterus; proliferative tissues had higher levels as opposed to secretory phase tissues. These studies were confirmed in the rat tissue.

Using steroid exchange assays, it was shown that estrogen treatment of rats increased the receptor levels; however, progestin treatment decreased the receptor level in a dose dependent manner. In detailed studies it was shown that progestins cause depletion of cytosolic receptors and also inhibit the nuclear receptor synthesis. In similar studies in human uterine tissues, a down regulation of both estrogen and progestin receptors was found following treatment of patients with norethindrone. In related work, it was shown that hormone mediated changes in receptor levels correlated with changes in RNA, DNA and protein. An early endometrial protein was characterized, whose synthesis was stimulated by estradiol, and inhibited by high doses of progestins. These studies are innovative and suggest a direct action of progestins in the human uterus by modulating its sensitivity to hormones.

Another aspect of the programme of research on hormone action has been an in-depth study on the human uterine nuclear receptor for progestins. By using reconstituted systems, Laumas and coworkers optimized conditions for receptor transformation and its subsequent translocation to the nucleus and binding to DNA. Further studies demonstrated that because of the distinct advantages of (a) binding with high affinity, (b) remaining unmetabolized, (c) less exchangeability, and (d) lack of non-selective binding to corticosteroid globulin, norethindrone, in particular, may serve as an excellent steroid for screening of potential anti-progestins. The above significant contributions towards the action of progestins have opened the doors for testing new anti-progestins for



fertility regulation and therapeutic hormonal treatments based on direct modulation of uterine sensitivity to hormones.

### **(3) Steroid Receptors in the Management of Breast Cancer**

Laumas and colleagues have made significant contributions to the study of estradiol and progesterone receptors in the normal and cancerous breast tissues. They have characterized these receptors and promoted their use for determining hormone dependence of breast tumours. Based upon this knowledge, a better management of the breast cancer patient is possible. The researches of Laumas are aimed at improving the usefulness of hormone receptor assays for their predictive value in determining hormone dependence of breast tumours.

### **(4) Steroid Metabolism**

Laumas provided evidence for the validation of the equation for the estimation of secretion rate and calculation of metabolic clearance rate (MCR) of steroids in man. He applied these concepts extensively to the determination of MCR of contraceptive steroids in women, thus gaining an understanding of their action and clearance from the body. Contraceptive steroids provide a useful method for fertility control during lactation; however, concern is expressed that these drugs may be excreted in the mother's milk and transmitted to the infant. The work of Laumas demonstrated for the first time that contraceptive steroids are excreted in the milk of lactating women and based upon extensive investigation it was found that low dose progestins would be better suited for control of fertility in lactating women.

### **(5) Intravas Nonocclusive Device for Control of Male Fertility**

There is need for a reversible technique for control of male fertility, which could overcome the problems associated with the wide use of vasectomy due to lack of reversibility in a number of cases. Laumas and colleagues have developed a nonocclusive intravas copper device for long-term male contraception. This device has given highly satisfactory results in rats and monkeys. The device has been further improved to incorporate silver in it. The copper-silver device is expected to serve as a long-term device for control of male fertility.

### **(6) Development of Reproductive Biology as a Discipline**

Laumas has made significant contributions to the development of reproductive biology as a discipline at the All India Institute of Medical Sciences, New Delhi. He has promoted the strengthening of this discipline in other medical colleges in the country. Reproductive biology is expanding and becoming a major discipline in its own right. Through training programmes and workshops, Laumas has strengthened this field. He has been instrumental in working with 33 different medical colleges in the country, sensitizing their faculties for the introduction of integrated teaching of human reproduction and family planning at the undergraduate level and thus reorienting the training of physicians in the country.

Through his efforts, the Department of Reproductive Biology, the first of its kind in India, was established. His contributions were recognized by the authorities by appointing him to the first chair created in

this discipline at the All India Institute of Medical Sciences.

Laumas was given the National Family Planning Award for the best research work in Reproductive Biomedicine in 1969-70. He organized as chairman and coordinator, the fifth international Congress on Hormonal Steroids, 29 October-4 November 1978, New Delhi. He has been invited to be chairman of many international symposia and to give orations about his contributions by different societies. He has been consultant with WHO and has served on steering committees of WHO task forces dealing with long-acting systemic agents for regulation of fertility and a task force on anti-implantation agents. He was President of the Endocrine Society of India. He has established a Hormone Foundation for the promotion of research on hormones and is its founder President. He is a member of the US Endocrine Society and a Fellow of the National Academy of Medical Sciences (India).

### Selected Publications

1. Kapur M M, Mokkapati S, Farooq A, Ashan R K & Laumas K R, Copper intravas device (IVD) and male contraception, *Contraception*, **29** (1984) 45-54.
2. Singh H, Uniyal J P, Jha P, Takkar D, Murugesan K, Hingorani V & Laumas K R, Pharmacokinetics of norethindrone acetate in women after the insertion of a single subdermal implant releasing norethindrone acetate, *Acta Endocr, Kbh*, **99** (1982) 302-8.
3. Kasid A & Laumas K R, Nuclear progestin receptors in human uterus, *Endocrinology*, **109** (1981) 553-60.
4. Laumas V, Jain A K, Jha P, Rahman S A, Kumar D, Malik B K, Sarkar N N, Takkar D, Hingorani V & Laumas K R, Correlation between serum norethindrone (Net) levels attained after insertion of a silastic implant releasing norethindrone acetate and the endogenous hormones, particularly progesterone, *Contraception*, **23** (1981) 211-25.
5. Verma U & Laumas K R, Screening of anti-progestins using *in vitro* human uterine progesterone receptor assay system, *J Steroid Biochem*, **14** (1981) 733-40.
6. Kasid A & Laumas K R, Progestin receptors in human uterine cytosol, *Molec cell Endocr*, **19** (1980) 131-42.
7. Singh H, Uniyal J P, Jha P, Murugesan K, Takkar D, Hingorani V & Laumas K R, Pharmacokinetics of norethindrone acetate in women, *Am J obstet Gynec*, **135** (1979) 409-14.
8. Laumas V, Malik B K, Jamal K, Seth U, Aggarwal N, Hingorani V & Laumas K R, Radioimmunoassay of norethindrone: Serum levels of norethindrone in lactating women after insertion of a single silastic implant releasing norethindrone acetate, *Contraception*, **186** (1978) 593-605.
9. Verma U, Kapur M M & Laumas K R, Characterization of progesterone receptors and metabolism of progesterone in the normal and cancerous human mammary gland, *J Steroid Biochem*, **9** (1978) 569-78.
10. Kasid A, Buckshee K, Hingorani V & Laumas K R, Interaction of progestins with receptors for progestins in the human uterus, *Biochem J*, **176** (1978) 531-39.
11. Verma U & Laumas K R, Cellular and sub-cellular metabolism of progesterone by the human proliferative and secretory phase endometrium and myometrium, *J Steroid Biochem*, **7** (1976) 275-82.
12. Uniyal J P & Laumas K R, Binding of norgestrel to human plasma proteins, *Biochim biophys Acta*, **427** (1976) 218-30.
13. Uniyal J P, Buckshee K, Sharma U & Laumas K R, Disappearance of (14, 15-<sup>3</sup>H) d, 1-norgestrel from plasma and its distribution in the reproductive tract of women, *Acta Endocr*, **82** (1976) 851-64.
14. Bhatnagar S, Srivastava U K, Takkar D, Chandra V L, Hingorani V & Laumas K R, Long-term contraception by steroid releasing implants. II. A preliminary report on long-term contraception by a single silastic implant



- containing norethindrone acetate (NETA) in women, *Contraception*, **11** (1975) 505-21.
15. Laumas K R, Malkani P K, Bhatnagar S & Laumas V, Radioactivity in the breast milk of lactating women after oral administration of H-norethynodrel, *Am J Obstet Gynec*, **98** (1967) 411-13.
16. Laumas K R & Farooq A, Uptake of estradiol 6, 7-  $^3\text{H}$  by brain and genital tract of rat, *J Endocr*, **36** (1966) 95-96.

## Usha K Luthra

During 1955-70, Luthra was a teacher and a researcher at Lady Hardinge Medical College, New Delhi, Sarojini Naidu Medical College, Agra and Harvard University, Boston, USA. From 1970 till date she has been a researcher and research administrator at the Indian Council of Medical Research, New Delhi, as Director of the Cytology Research Centre and Senior Deputy Director-General, incharge of the Division of Non-communicable Diseases at ICMR Headquarters.

### Research Contribution

During the last two decades, Luthra has made outstanding contributions in the field of basic and applied oncology for the two most commonly encountered cancers in India, viz. oral cancer and cervical cancer. Her studies on these two cancers have followed a comprehensive approach, starting with descriptive and analytic epidemiology, experimental carcinogenesis, natural history and biological behaviour followed by intervention strategies utilizing the knowledge gained for their prevention and control.

Simultaneously, she has been actively involved in education and human resource development in the specialized field of cytology through formal and informal training programmes.

Epidemiological studies on oral cancer in Mainpuri District in UP revealed Mainpuri as having the highest incidence of oral cancer in the world, i.e. 21 per 100,000 population and establishing for the first time the role of Mainpuri tobacco in relation to oral cancer. Her experimental studies using Syrian golden hamsters with a known carcinogen established the cytomorphological alteration during the process of oral carcinogenesis. She succeeded in producing in mice and rats oral precancerous lesions with Mainpuri tobacco and its extracts. Recently, she has been involved with the planning and evolution of a feasibility module for primary and secondary prevention of oral cancer in Goa, Bangalore and Mainpuri District, utilizing the existing health care infrastructure.

Her studies on cervical precancerous and cancerous lesions have yielded significant results which have far reaching consequences in prevention and control of this important cancer in Indian women. For this cancer again, she followed a systematic approach starting with descriptive and analytic epidemiology, inducing cervical precancerous and cancerous lesions in mice, multidisciplinary approach to study the uterine cervix dysplasia with a view to understanding their biological behaviour and natural



history; currently she is busy evolving at the Cytology Research Centre, in collaboration with Maulana Azad Medical College, New Delhi, a feasibility module for community control of cancerous lesions of the uterine cervix through secondary prevention utilizing the existing primary health care services.

Her studies have highlighted the important epidemiological risk factors for cervical cancer in Indian women like early age of intercourse, multiparity, personal hygiene and role of Herpes Simplex type II virus. Currently, she is working on the role of human papilloma viruses in relation to cervical carcinogenesis. Her experimental studies in mice helped her to characterize the cytomorphological alteration induced during chemical cervical carcinogenesis with clearcut cytological recognition of precancerous states. Her multidisciplinary study on uterine cervical dysplasia currently in progress at the Cytology Research Centre is probably the most comprehensive study being undertaken in the South East Asian region if not the world to gain insight into the natural history and biological behaviour of cervical cancer with the ultimate aim of its prevention and containment. Important leads have emerged from her work at the Cytology Research Centre on the natural history of cervical cancer, which have already found application in the National Cancer Control Programme for secondary prevention of cancer of the uterine cervix, the commonest cancer in Indian women. She is aptly utilizing the knowledge gained from her laboratory studies through operational research to field studies by evolving a feasibility module for secondary prevention of cervical cancerous lesions using the existing primary health care services.

### **Human Resource Development**

As Director of the Cytology Research Centre and Chairperson of the Indian Academy of Cytologists' Standing Committee on Accreditation and Examination for Cytology, she has been responsible to a large extent for initiating and establishing a national grid of trained personnel in this specialized field of biomedicine through the conduct of workshops, service programmes, etc. As of present, 16 laboratories/centres have been accredited by the Academy for diagnostic cytology, and 5 of these also for training.

### **Research Management**

As Chief of the Division of Noncommunicable Diseases, Luthra has been responsible for planning, initiating and monitoring research in this field in the country and through bilateral programmes in other countries. Major areas for such programmes are cancer, cardiovascular diseases, mental health, blindness, occupational and environmental health, oral health, liver diseases, neurological disorders, etc. During early seventies, major efforts were made in initiating multicentred studies to get prevalence data and epidemiological profiles of major noncommunicable diseases. Realizing these as major public health problems, intervention studies have been initiated for some of them like oral and cervical cancers, rheumatic fever and rheumatic heart disease, drug abuse, etc. using the existing health care infrastructure. Studies in other areas are also being planned. This strategy is in keeping with the goal of 'Health for all by 2000 AD'.

## Future Direction

At the Cytology Research Centre, it is proposed to continue the recently initiated ongoing studies with the ultimate goal of control and prevention of cancer of the uterine cervix. Studies on breast cancer, with special reference to early detection through breast self-examination, through paramedical personnel and confirmed by physicians, and in-depth and follow-up studies on the so-called "precancerous lesions" of the breast in order to understand the natural history of breast cancer, would be initiated.

Studies on lymphomas and leukaemias, with special reference to their early diagnosis, characterization and management, are also proposed to be undertaken.

Human resource development, possibly through a well planned School of Cytotechnology, is an urgent need for the country and would be undertaken.

At the Indian Council of Medical Research Headquarters, as Chief of the Division of Noncommunicable Diseases, she plans to continue her efforts to contain the problem of NCD using the existing knowledge and discovery of new knowledge through universities and medical institutions. Establishing a chain of advanced centres for long-term research in selected areas of NCD around scientists of excellence would be another approach towards achieving the goal of containing these problems.

## Selected Publications

1. Murthy V V S, Mitra A B & Luthra U K, Activity of nucleolar organizer regions (NORs) in leukocytes in women with cervical precancerous and cancerous lesions, *Am J hum Gen*, **36**(4) (Suppl) (1984) 34(s).
2. Luthra Usha K, Mitra A B, Prabhakar A K, Agarwal S S & Bhatnagar P, Cytological monitoring of women using copper containing intrauterine devices—Five years follow up study, *Acta Cytol*, **20**(5) (Sept-Oct 1982) 619-22.
3. Mitra A B, Murthy V V S & Luthra U K, Sister chromatid exchanges in leukocytes in patients with cancer of uterine cervix, *Hum Genet*, **60** (1982) 214-15.
4. Luthra Usha K, Mitra A B, Prabhakar A K, Bhatnagar P & Agarwal S S, Role of copper containing intrauterine devices in cervical carcinogenesis, *Indian J med Res*, **72** (1980) 659-64.
5. Luthra Usha K, Problem of carcinoma of cervix, *Ann Indian Acad med Sci*, **12** (1977) 1-5.
6. Luthra Usha K, Epidemiology of cervical cancer in India: Cancer in Asia—Opportunities for prevention, detection & treatment, *GANN, Monograph on Cancer Res*, No. 18, Japanese Cancer Ass, University of Tokyo, 1976, 161-66.
7. Wahi P N, Cohen B & Luthra Usha K, International histological classification of tumour, Histological typing of oral and oropharyngeal tumour, *World Health Organization Monograph No. 4*, 1971.
8. Luthra Usha K, Pande R N, Prabhakar A K & Kamala Rani, Epidemiology and distribution of cancer of uterine cervix in an urban population of Delhi-India: Liver cancer—Cancer problems in Asian countries, *Proc AFOCC 2nd Asian Cancer Conf*, Sept 22-25 '75, Singapore, 443-49.
9. Luthra Usha K, Woods D A, Wahi P N & Gupta Manorama, Ultrastructure of human oral mucosa after prolonged exposure to tobacco, *Indian J med Res*, **59** (1971) 157-62.
10. Luthra Usha K, Natural history of cancer of the uterine cervix—Its significance in prevention and control of this tumour, Dr P N Raju Oration Award, 1970, *Indian J med Res*, **58** (1970) 805-28.
11. Luthra Usha K, Mali Saraswati & Wahi P N, Cervical dysplasia—Its significance. III: Biological behaviour, *Indian J med Res*, **57** (1969) 629-41.
12. Mali Saraswati, Luthra Usha K & Wahi P N, Cervical dysplasia—Its significance. II. Clinical and epidemiological studies, *Indian J med Res*, **57** (1969) 624-28.
13. Wahi P N, Luthra Usha K & Mali S, Cervical dysplasia—Its significance. I. Cytomorphological studies, *Indian J med Res*, **57** (1969) 617-23.
14. Luthra Usha K, Bhardwaj V P, Lahiri V L & Wahi P N, Succinic dehydrogenase activity in DMBA induced experimental oral carcinogenesis



- in hamster cheek pouch, *Indian J med Res*, **56** (1968) 1766-70.
15. Kehar Usha & Wahi P N, Cytologic and histologic behaviour patterns of the premalignant lesions of the cervix in experimentally induced cervical dysplasia, *Acta Cytol*, **11** (1967) 1-15.
16. Wahi P N, Luthra Usha K & Kapur V L, Submucous fibrosis of the oral cavity. Histomorphological studies. (Abst. in EENT Digest (Oct 1967) p 82, also abstracted in oral Res Abst 2(9) 758, Sept 1967) *Br J Cancer*, **20** (1966) 676-87.
17. Wahi P N, Kehar Usha & Lahiri B, Factor influencing oral and oropharyngeal cancer in India, *Br J Cancer*, **19** (1965) 642-60.
18. Wahi P N, Lahiri B, Kehar Usha & Arora, Shashi, Oral and oropharyngeal cancer in North India, *Br J Cancer*, **19** (1965) 627-41.
19. Luthra Usha K & Wahi P N, Intra-epithelial carcinoma of the uterine cervix, *Extrait De Acta Union Internationale Contra Le Cancer*, **19** (1963) 1379-83.
20. Wahi P N, Luthra Usha K, Mali S & Misra G D, Histopathogenesis of carcinoma *in-situ* of the uterine cervix (awarded Shakuntala Amir Chand Prize for best research publication, 1962) *Bull Wld Hlth Org*, **26** (1962) 661-74.

## K S Mathur

Mathur has distinguished himself as a medical scientist, an eminent teacher and educationist. He has published 272 papers in Indian and foreign journals and authored chapters on cardiovascular and kidney diseases in several textbooks of medicine. His contributions in the field of medical sciences in general and cardiology in particular have received national and international recognition; he is recognized as one of the pioneers in experimental cardiology in the country.

The important contributions of Mathur relate to a series of studies dealing with epidemiological, experimental and biochemical facets of atherosclerosis, coronary heart disease, rheumatic heart disease and nephritis.

Based on a study of 900 medicolegal autopsies, Mathur has shown the progression of atherosclerosis in our population in various age groups and has broken new ground in this area<sup>1,2</sup>.

The role of vessel wall in pathogenesis of atherosclerosis has been suggested through the demonstration of correlation of atherosclerosis with the lipid-binding and cholesterol-binding capacities of the globulins in the absence of any correlation between atherosclerosis and blood cholesterol or total globulin content in autopsy studies<sup>3</sup>.

Epidemiological studies of Mathur on coronary heart disease gave a prevalence of 5.3% in the adult population of Agra and serum cholesterol above 200 mg as the most significant risk factor. An exciting observation was the low levels of serum cholesterol and coronary heart disease in sections of population whose staple diet is Bengalgram<sup>4</sup>. This led to a series of experiments on animals and human volunteers for evaluating the value of Bengalgram in affecting the cholesterol levels in blood. One section of the population previously surveyed living under similar cultural, religious and dietetic conditions in Dayalbagh and Swamibagh area, has been revisited after 20 years; the results are being analysed with the help of the computer.

Hypocholestrolemic<sup>5</sup> and anti-atherogenic<sup>6</sup> actions of Bengalgram have been demonstrated through a series of animal experiments. The results of animal experiments led to work on dietary manipulation on human volunteers showing more than 15% reduction in serum cholesterol in 16 of the 20 subjects and its sustained action on long-term administration<sup>7</sup>. The mode of action is still not clear and work is continuing in many laboratories. Since hypercholesterolemia is one of the most important risk factors in coronary heart disease, its inclusion in the

---

Professor Emeritus, Post-Graduate Department of Medicine, Sarojini Naidu Medical College, Agra; Residence : 1/130 Professors' Colony, Agra-282002.



diet has been suggested as a practical and safe method rather than the administration of costly and harmful drugs for the prevention of coronary heart disease. A change in dietary way of life since childhood would help in the prevention of coronary heart disease, since atherosclerosis is the basis of all coronary episodes.

The hypocholesterolemic action of unsaturated fats was confirmed in a series of experiments on animals as well as human volunteers<sup>8</sup>. A significant contribution was the demonstration of a combination of saturated and unsaturated fats in the ratio 1 : 1 as having the same hypocholesterolemic action as unsaturated fats alone. This is of practical importance for those whose life-long habit does not allow them to change over to oil completely.

Studies on experimental infarction in dogs following coronary artery ligations at various levels and localized myocardial damage by formalin have advanced our knowledge of myocardial infarction and the uncommon electrocardiographic patterns met within this condition.

Epidemiological studies on rheumatic heart disease covering 29,922 urban school children at Agra giving a prevalence of 5.1 per 1000<sup>9</sup> and house to house survey of rural population of Bichpuri Block of villages with a prevalence rate of 2 per 1000 in subjects below 30 years<sup>10</sup> brings out the high prevalence of rheumatic heart disease in this area and highlights certain etiological factors.

The role of autoimmunity in rheumatic heart disease has been suggested by a study of circulating auto-antibodies by tanned red cell passive haemagglutination

technique as well as by indirect fluorescence technique in 400 cases of rheumatic fever and rheumatic heart disease and their correlation with rheumatic activity and organ specific symptomatology<sup>11</sup>.

Re-evaluation of Duckett-Jones criteria has been undertaken by subjecting clinical and laboratory investigations to multivariate discriminant function analysis with the help of the computer. It has been suggested that polyarthralgia and electrocardiographic changes be accepted as major manifestations in addition to other known parameters for diagnosis of rheumatic activity<sup>12</sup>.

Renal biopsies of patients and experimentally induced nephropathy in animals have contributed to the study of natural history of nephritis<sup>13</sup> and the evaluation of immuno-allergic concept in its etiology<sup>14</sup>.

The awards won by Mathur include the Watumull Memorial Award of Watumull Foundation; Award for Experimental Medicine of the Association of Physicians of India; Chaturvedi Kalawati Jagmohan Memorial Award of the Indian Council of Medical Research; Dr B C Roy National Award of the Medical Council of India; and Dr Jal Rustom Vakil Memorial Award of the All India Heart Foundation.

### Selected Publications

1. Mathur K S, Patney N L & Kumar V, Atherosclerosis in India—An autopsy study of the aorta and the coronary, cerebral, renal and pulmonary arteries, *Circulation*, **24** (1961) 68.
2. Mathur K S, Kashyap S K & Mathur S C, Distribution and severity of atherosclerosis of aorta, coronary and cerebral arteries in persons dying without morphological evidence in Northern India—Study of 900 autopsies, *J Ass Phys India*, **16** (1968) 113.

3. Mathur K S, Sharma R D, Kashyap S K & Sapru R P, Lipid binding capacity of intimal globulins in Human Aortic Atherosclerosis, *Circulation*, **30** (1964) 694.
4. Mathur K S, Wahi P N, Gahlaut D S, Sharma R D & Srivastava S K, Prevalence of coronary heart disease in general population at Agra, *Indian J med Res*, **49** (1961) 605.
5. Mathur K S, Singhal S S & Sharma R D, Effect of Bengalgram on experimentally induced high levels of cholesterol in tissue and serum in albino rats, *J Nutr*, **8**(4) (1964) 201.
6. Mathur K S, Wahi P N, Lahiri B, Sharma R D & Jain S C, Effect of Bengalgram on cholesterol induced atherosclerosis in rabbits, *J Indian med Ass*, **58** (1970) 25.
7. Mathur K S, Khan M A & Sharma R D, Hypocholesterolemic effect of Bengalgram on serum lipids and fecal elimination of cholesterol and products: A long term study on human subjects, *Br med J*, **6** (1968) 30.
8. Mathur K S, Tandon K K & Sharma R D, The influence of dietary fats on serum lipids and fecal excretion of bile acids: A long term study on human volunteers, *Indian J med Res*, **52** (1964) 470.
9. Mathur K S & Wahal P K, Epidemiology of rheumatic heart disease—A study of 29,922 school children, *Indian Heart J*, **34** (1982) 361.
10. Mathur K S, Rheumatic fever and rheumatic heart disease—Its prevalence in a village community of Bichpuri Block, Agra, *J Indian med Ass*, **19** (1971) 151.
11. Mathur K S, Wahi P N, Wahal P K, Atal P R, Kumari J & Hazra D K, Auto antibodies in rheumatic fever and rheumatic heart disease and their correlation with rheumatic activity and organ specific symptomatology, *Indian Heart J*, **21** (1968) 154.
12. Mathur K S, Diagnosis of recurrence of rheumatic fever—A re-evaluation of Duckett-Jones criteria, *Indian J med Res*, **64** (1976) 1634.
13. Mathur K S, Wahi P N, Kehar U, Gulati P D & Brij Kishore, Natural history of nephritis—An experimental study, *Indian J med Res*, **50** (1962) 354.
14. Mathur K S, Wahi P N, Kehar U, Brij Kishore & Gulati P D, Immuno-allergic concept of nephritis, *Indian J med Res*, **17** (1963) 121.



## R K Pal

Pal has made significant contributions mainly on endocrines and nutrition.

(1) In one of his early papers<sup>1</sup>, he described the structure of a persistent thyroglossal duct. An interesting feature of the observation is the continued differentiation of the thyroglossal duct until it resembles the alimentary canal with convoluted epithelium, tubular glands in the submucosa, internal and external muscular coats and the most external fibrous tunic. Presence in the neighbourhood of an aberrant thyroid-like glandular mass in the course of the persistent duct confirms the diagnosis.

(2) A small dose of insulin added to the perfusion fluid of a frog's heart was found to slow the number of beats and a big dose to cause a 'vagus escape'-like phenomenon (temporary stoppage of the heart with automatic resuscitation later)<sup>2</sup>. The blood pressure was also lowered. As these effects can be counteracted by a previous injection of atropine, it has been definitely proved that insulin has a stimulating action on the parasympathetic nerve endings.

This vago-tropic action is now utilized for obtaining more pyloric acid secretion for differential diagnosis by analysis of the acid content with the presence of lactic acid for malignancy. Similarly, in the treatment of diabetes mellitus with very high blood sugar content requiring a big

dose of insulin one should guard against a heavy fall in blood pressure by taking appropriate measures.

(3) It was found that different sugars increase the efficiency of heart contractions when added in suitable doses to the perfusion fluid of frog's heart. In this respect, though glucose as a rule heads the list, other mono- and disaccharides are also worth trying. Insulin enhances further the action of the sugars<sup>3</sup>. As pH of the perfusion fluid is lowered in the course of perfusion, a temporary dilatation of the heart with diminution in amplitude is noticed, to be followed by true augmentation. The effects of insulin stimulating the vagus are absent or rarely present when a suitable dose of insulin is combined with suitable doses of different sugars. Intravenous injection of glucose preceded by a small dose of insulin, injected hypodermically some time previously, acts as a very good cardiac tonic.

(4) It was found that Lugol's iodine solution lowers the rate but increases the amplitude of contraction of a normal heart (frog's), while thyroxine in small doses causes a slight increase in the rate with diminished auricular complex; but when the hormone is allowed to act for some time, the heart becomes irregular with grouped beats<sup>4</sup>. Lugol's iodine can remove

---

Formerly, Vice-Principal and Professor of Physiology, RG Kar Medical College, Calcutta; Residence : 5/4, Ballygunge Place, Calcutta-700019.

completely this toxic effect and make the action of the heart regular. The potassium content of Lugol's iodine is not responsible for this beneficial action.

It was concluded that Lugol's iodine treatment in hyperthyroidism (thyrotoxicosis) improves the condition not only by lowering the basal metabolism (the mode of action is obscure) but also by counteracting the toxic effect of the hypersecretion of the thyroid on the heart to a considerable extent.

(5) In one study<sup>5</sup>, perfusion fluid from one frog's heart was allowed to pass to a reservoir from which the same fluid was again perfused through another frog's heart simultaneously and records of both hearts' contractions were taken, one above and the other below. If drugs like nicotine acting on the ganglia, atropine and physostigmine acting on the vagus terminals or adrenaline acting on the sympathetic endings are dropped on the perfused heart with immediate action on the first heart, an exactly similar action is noticed on the second heart, showing that in each case the effect on the second heart is due to humoral transmission.

(6) Vitamins A, B1 and C were found to have a profound effect in maintaining the healthy condition of the secreting cells of the parathyroid glands; vitamin D, on the other hand, appears to promote a resting phase of the glands, in the absence of which the principal cells become hypersecreting, indicating that vitamin D is also essential for the normal action of the glands<sup>6</sup>.

(7) Ordinarily, the average South Indian diet is nutritionally poor<sup>7,8</sup>. Rats on such a diet were given in addition calcium as calcium lactate, sodium phosphate and

calcium phosphate (as a combination of both calcium and phosphorus); after two months' feeding those getting the first and the third supplement showed considerable improvement in growth (as shown by increase in weight), but the supplement of sodium phosphate alone had practically no effect. This was also corroborated by measurement of their lengths (including tails) as also post-mortem measurement of femurs with increased calcium contents of the same bones by vital staining, where externally growth was manifested. This definitely proves that the diet of the South Indians is very much deficient in both calcium and phosphorus, indicating the need for more intake of milk, if possible; otherwise, mineral salts such as calcium phosphate or even calcium lactate may be of some help.

(8) A heifer showing some unnatural bullish characters was given injections of PUH and ovarian steroidal hormones for two months; with this, not only did the bullish nature disappear, but the udder with the teats was swollen, yielding about a litre of milk in the morning and evening<sup>9</sup>. This milk was analysed daily for ten days and compared with the ordinary milk of milch cows. Milk from the uncovered heifer was found to be slightly richer in total solids, protein, albumen, phosphorus and iron contents, but it was somewhat poorer in casein than the average normal milk. So, the nutritive value of this particular milk is in no way inferior to that of the average milk; in fact, it appears to be slightly better.

(9) A very high pulse diet based largely on cereals must not form the diet of the poor people who cannot afford to take sufficient quantity of *ghee* (clarified butter), fresh butter or milk<sup>10</sup>. Small quantities of



yeast, cod liver oil, green vegetables like carrot and radiostol (BDH) prepared from yeast may also be used as adequate supplement to prevent damage to the kidneys and liver, probably caused by an unbalanced diet due to high amino acid components of the pulses. The preventive substance is thermostable and is probably quite distinct from any other known accessory food factor or vitamin, for which a tentative nomenclature of anti-aminotoxic factor (A-A-T factor) is suggested.

Some more work is necessary to establish the essential nature of this factor.

(10) In another study<sup>11</sup>, it was found that *l*-tyrosine has no supplementing effect on ordinary Bengalee diet and its addition to the diet provides no indication that the amino acid is needed for growth and nitrogen balance is also not affected thereby. But most probably it is indispensable in so far as the secretion of thyroid gland is concerned. The deficiency of this amino acid leads to pathological changes (hyperplasia with fibrosis) in the gland.

(11) From a comparative study on the compositions of crops (Bengal gram) grown under different climatic and soil conditions, protective principles such as protein and calcium were found to be higher in Delhi samples<sup>12</sup>. There was no difference in the energy forming components (fats and carbohydrates) in the samples grown in Delhi and Pussa. The moisture, phosphorus and iron contents were more in Pussa samples. Regarding the total mineral matters, the increase in calcium in the first sample was probably well compensated by lowering of the phosphorus, iron, etc. and vice versa in the Pussa sample. So, there is no

significant difference in the total mineral contents of the two samples grown under different soil and climatic conditions. The Karnal sample was similar to the Delhi sample as regards protein and calcium contents but those of phosphorus and iron were higher compared to the Delhi or Pussa samples. So, climate and soil conditions play important roles in altering the nutritive values of crops.

(12) Some of the 'sulphonamides, such as Prontosil, Dagenan sodium, Soluseptasine, etc. have a temporary stimulating action upon the heart, but all of them when administered for considerable period show some toxic effects on the heart, such as irregularity of heart beat, grouped beats, extreme slowing and sometimes complete stoppage<sup>13</sup>. Their action is partly on the autonomic ganglia (stimulation), but mainly on the muscles of the heart. On small doses there is no appreciable effect on the carotid pressure but on bigger doses there is a fall, Dagenan sodium producing more marked effects than Soluseptasine or sulphonamides soluble. Sodium bicarbonate and acetate prevent and also eliminate the deleterious effects. Sometimes when administered together with the sulphonamide, the action on the heart is better. It was concluded that sodium bicarbonate or acetate should always be given if sulphonamides are administered.

(13) Radio-iodine (<sup>131</sup>I) practically has no effect on any other part of the body except on the thyroid gland specifically and blood picture to some extent<sup>14</sup>. The thyroid structure undergoes fibrosis and degeneration without any evidence of regeneration as a result of the uptake of the radio-iodine specifically by the gland and erythrocytic and leucocytic total

counts go down due to the destruction of some of the corpuscles by the beta-radiation from the isotope circulating in the blood. It was concluded that since there is no deleterious effect on any other organ or tissue, radio-iodine therapy may be undertaken without any hazard in thyrotoxicosis. A case of very successful treatment in an obstinate case of Grave's Disease was recorded<sup>15</sup>.

(14) It was found that muscle meat causes very slight or no increase in the uric acid content of blood. Beverages like tea, coffee, etc. do not usually raise the same, as increased production of uric acid is coped with by increased excretion of allantoin (by dog) owing to the increased diuresis by methyl purines contained therein<sup>16</sup>. Although large fish-containing food causes no increase of uric acid in blood, small fish must not be taken in excess or frequently by persons with gouty diathesis, as it is sure to raise uric acid in the blood.

Uric acid content of dog's blood is usually much lower than in man. With intravenous injection of uric acid solution (in sodium bicarbonate solution), as its concentration goes higher, gradually allantoin excretion in the urine diminishes progressively. At a particular concentration of the former in the blood with the least concentration of allantoin in the urine, there is haemorrhage from the gastro-intestinal mucosa, which can be checked or prevented by immediate elimination of more uric acid with the help of some urosuric drug like colchicum, salicylates or cincophen.

Although colchicum, salicylates and cincophen are all capable of lowering the uric acid content of the blood by

promoting excretion of more allantoin, they are not equally effective. Sodium salicylate appears to be more useful than cincophen or colchicum and combination of the two is no better than one of them alone. Of the different pharmacological preparations of colchicum, the tincture is probably better than the vinum, although both of them are credited with relief of pain in gout (acute). Cincophen, on the other hand, is very effective initially in diminishing the uric acid content of the blood by increased elimination, but this effect lasts only for 48 hours or so.

(15) Two new hypoglycaemic drugs, N'-p-sulphonamidophenyl guanyl urea hydrochloride (SGU) and N'-p-sulphonamidophenyl biguanide hydrochloride (SBG) were prepared and tried on normal and experimental (alloxanized and partly depancreatized) diabetic dogs and also on some uncomplicated and complicated diabetic patients<sup>17</sup>. The effects of these drugs were compared with those of other known oral hypoglycaemic drugs from various standpoints, such as lowering of the blood sugar, stimulation of glucose uptake and glycogen synthesis by muscles of the diaphragm, blood chemistry and that of urine as also histological study of the pancreas and some other organs of the body. On the whole, of the two new drugs, SGU appears to be better than SBG and equally effective, if not better in some respects than other potent sulphonylurea drugs like Rastinon and Nadison. SBG is, however, more or less like DBI, another biguanide preparation. No toxic effect was noted with SGU and as such it may find a place among the potent orally effective hypoglycaemic drugs for the treatment of various types of diabetes mellitus, adult, juvenile, uncomplicated and complicated.



### Selected Publications

1. Pal R K, Persistent thyroglossal duct in a rabbit, *J Anat*, **63** (Pt III) (1934).
2. Pal R K & Prasad S, Action of insulin on heart and blood pressure, *J Physiol*, **82** (1934) 154.
3. Pal R K & Prasad S, A comparative study of the effect of adding different sugars to the perfusion fluid through the frog's heart and the influence of insulin over it, *J Physiol*, **83**(3) (1935) 285.
4. Pal R K & Prasad S, Action of Lugol's iodine solution on the thyroxinised heart, *Indian J med Res*, **23**(4) (1936).
5. Pal R K & Prasad S, Humoral transmission of the effects of cardiac vague and sympathetic stimulation by drugs, *Indian J med Res*, **24** (1936) 262.
6. Pal R K, Structural changes in the parathyroids in vitamin deficiencies, *Indian med Gaz*, **72**(10) (1937) 593.
7. Pal R K & Singh N, Effects on rats of supplementing a South Indian diet with calcium and phosphorus, *Indian J med Res*, **25** (1938) 693.
8. Pal R K & Singh N, Further studies on the effects on rats of supplementing a South Indian diet with calcium and phosphorus, *Indian J med Res*, **25** (1938) 754.
9. Pal R K & Bose N M, Nutritive value of milk from an uncovered heifer, *J Ped*, **9**(35) (1942) 141.
10. Pal R K & Bose N M, The position of pulses in a diet based largely on cereals, *Indian med Gaz*, **78**(9) (1943) 436.
11. Pal R K & Bose N M, Effects on rats of supplementing a Bangalee diet with *l*-tyrosine, *Ann Biochem exp Med*, **9**(2) (1943) 99.
12. Pal R K & Bose N M, Variations in the composition of crops (pulses) grown in different areas under varying conditions of soil and climate, *Ann Biochem exp Med*, **5**(1) (1944) 25.
13. Pal R K, Action of sulphonamide on heart and blood pressure, *Indian J Physiol all Sci*, **1 & 2** (1947) 25.
14. Pal R K & Mitra B N, Effects of radio-iodine on blood and some important organs, *Indian J Physiol all Sci*, **4**(2) (1950) 84.
15. Pal R K, Sen S K & Chatterjee, Treatment of Graves disease with radio-iodine, *Heart J*, **2** (1950) 117.
16. Pal R K & Mitra B N, Studies in uric acid metabolism, *Indian J Physiol all Sci*, **8**(3) (1954) 89.
17. Pal R K, Poddar S N & Mitra B N, A comparative study of the effects of two new hypoglycaemic drugs and those of other effective ones already in use on the normal and experimental diabetic dogs as also a few cases of human diabetes mellitus, *Proc natn Inst Sci India*, **31B**(3,4) (1965) 129.
18. Pal R K, *A handbook of modern physiology* (Oxford & IBH) 5th edn, 1970.
19. Pal R K, *Biology of senescence: Problems and perspectives* (Asiatic Society) 1972.

## T Ramakrishnan

The genus *Mycobacterium* belongs to the order Actinomycetales, a group of fungus-like bacteria and includes in its family, in addition to *Mycobacterium tuberculosis* (*M. tuberculosis*), *M. leprae*, the causative organism of leprosy, *M. smegmatis*, a non-pathogenic saprophytic organism, etc.

When work on *M. tuberculosis* H<sub>37</sub>R<sub>v</sub> and other mycobacteria was started in 1957 in our laboratory, the aims of the programme were three-fold :

(1) To study the enzyme systems of the virulent tubercle bacilli and compare them with those reported in the human host, so that any differences between them could be exploited for possible chemotherapeutic attack against tuberculosis.

(2) To compare the enzyme systems of the drug-sensitive and drug-resistant strains of the tubercle bacilli and to study the genetics of resistance to drugs like isoniazid and streptomycin, in order to combat the high incidence of drug resistance in India.

(3) To compare the enzyme systems and genetic make-up of the virulent and avirulent tubercle bacilli to find out the possible cause of virulence.

The work carried out by our group at the Indian Institute of Science has been covered in a review<sup>1</sup> dealing with the

metabolism and genetics of mycobacteria in general. A summary of the research carried out during the last 28 years (1957-1985) is given in what follows.

(1) Studies on the metabolic response of tubercle bacilli to oxygen showed that contrary to the conventional belief that tubercle bacilli are strict aerobes, the virulent strains of tubercle bacilli possess a functional glycolytic system<sup>2</sup>. Of utmost interest was the observation of Ramakrishnan *et al.*<sup>3</sup> who studied the relative extents to which two strains H<sub>37</sub>R<sub>v</sub> and H<sub>37</sub>R<sub>a</sub> dissimilated glucose by the glycolytic and oxidative pathways. It was found that the virulent strain dissimilated glucose by the glycolytic pathway to a greater extent than the avirulent strain. This metabolic property of the virulent strain might account for the fact that it can multiply and survive in the host more successfully than the avirulent strain, even in increasingly anaerobic conditions of the developing lesions.

*M. tuberculosis* H<sub>37</sub>R<sub>v</sub> was also found<sup>4</sup> to have all the enzymes of the tricarboxylic acid cycle and the glyoxylate bypass.

(2) To understand the role played by the nicotinamide adenine nucleotides in the oxidative metabolism of *M. tuberculosis* described above, a study of these coenzymes was undertaken. The

---

Formerly, Professor & Chairman, Microbiology & Cell Biology Laboratory, Indian Institute of Science, Bangalore-560012; Residence : 71, KHBCS Layout, West of Chord Road, Bangalore-560079.



organism was found to possess a nicotinamide adenine dinucleotide glycohydrolase (NADase) and also its protein inhibitor, which under normal conditions prevents the NADase from being active<sup>5</sup>. The isolation of INH resistant mutants by Sriprakash and Ramakrishnan<sup>6</sup> showed that in some of the resistant strains, the inhibitor was still sensitive to INH. It was also shown that during the mutation from INH sensitivity to INH resistance three proteins—catalase, peroxidase and Y enzyme—were altered at the same time<sup>7</sup>. During purification of these proteins from the crude extract of the bacillus, the same fold purification was found for all of them, suggesting that mutation affects one protein with three different activities. In the sensitive strains, INH is converted by this protein in the presence of NAD to a toxic compound, which inhibits the growth of *M. tuberculosis*. Though this compound has not been purified and identified, the molecular weight of the enzymatic product has been determined with the help of Sephadex gels and a tentative formula assigned to it. We hope to synthesize this compound chemically and determine whether INH resistant strains of the bacilli are inhibited by it.

It was during this stage that we had the good fortune to discuss the problem of drug resistance and other facets of the work on tubercle bacilli with Jacques Monod when he visited India in 1961. Monod had just then published his classical paper on the *lac* operon and he suggested that only the application of the then emerging science of molecular biology to the problem would lead to its solution. Some experience in this science was gained by Ramakrishnan while working for two years at Yale University

on the isoleucine-valine operon of *E. coli*. On his return to Bangalore in 1964, work on the molecular biology of tubercle bacillus was started.

In order to work on the molecular biology of *M. tuberculosis*, an inducible or repressible system in this organism was required, as well as a genetic system like conjugation, transduction or transformation to transfer genes from one mutant strain of the organism to another. Since none of these was available in this organism, we set out first of all to find such systems.

(3) Since *M. tuberculosis* uses L-asparagine as a preferred source of nitrogen in its growth medium, we first concentrated our attention on L-asparaginase in the organism. Metabolic differences were found between the avirulent and virulent strains of tubercle bacilli in the metabolism of L-asparagine by the two strains<sup>8,9</sup>. The avirulent strain was found to have inhibitory activity against Ehrlich ascites tumours. Since the DNA of *M. tuberculosis* is highly GC-rich, it would be of interest to sequence the promoter of the inducible L-asparaginase in this organism to see whether it is still AT-rich as in other organisms or not.

(4) The virulent strain had all the enzymes of the isoleucine-valine biosynthesis<sup>10</sup>. One of the interesting side-products of this study is that tubercle bacilli require vitamin C specifically for an essential enzymatic step in the biosynthesis of these branched amino acids. This is the first time that vitamin C has been shown to act as a coenzyme, and this finding has simplified the bioassay of this vitamin and its analogues, for which previously only tedious animal experimentation, using scorbutic guinea-pigs, was available.

(5) Since the phenomena of induction and repression in bacteria have been shown to act at the level of transcription, the biochemical aspect of transcription was studied in *M. tuberculosis*. Investigations on the RNA metabolism of the bacillus have shown<sup>11</sup> that the RNA polymerase of this organism has properties different from those of the polymerase of *E. coli*. It is 1000 times more sensitive to the antitubercular drug rifampicin than that of *E. coli*. To find out the reason for this, both the enzymes were incubated with labelled rifampicin and an attempt was made to leach out the label with cold rifampicin. The label was leached out from the mycobacterial enzyme much more slowly than from the *E. coli* enzyme. These experiments were carried out with the mycobacterial enzyme purified to homogeneity. Thus, a drug like rifampicin which acts at low concentrations on the RNA polymerase of *M. tuberculosis* not only does not affect the human host, whose enzyme is different from prokaryotic RNA polymerase, but also the normal flora of the human intestine, like *E. coli*, at these low concentrations. Based on this finding, a new potent antitubercular drug, N-naphthyl glycine hydrazine, has been developed<sup>12</sup>. This drug, which inhibits RNA polymerase of *M. tuberculosis* at low concentrations, in turn, inhibits the growth of the bacilli *in vitro* at 1 µg/ml. Dr P R Mahadevan, in a personal communication to Ramakrishnan has reported that the compound also showed anti-*Mycobacterium leprae* activity when it was tested using labelled dihydroxyphenylalanine (DOPA). The drug is now undergoing trials against experimental tuberculosis in guineapigs at the Patel Chest Institute, Delhi.

(6) Metabolic differences between the virulent and avirulent strains of *M.*

*tuberculosis* have also been found in the methylation of the bases in their DNA<sup>13</sup>. 5-Methylcytosine has been found only in the virulent strain and in *M. smegmatis* lysogenized by a phage. One of the possible conclusions from this work is that the virulence of the tubercle bacillus may be coded by a plasmid. Electron microscopic studies support the presence of such a plasmid in the virulent strain of *M. tuberculosis*, and no such plasmid can be demonstrated in the avirulent strain.

(7) The analysis of methylated bases in the transfer RNA of the organism revealed that it contains 1-methyl adenine and lacks ribothymidine. In this respect, it differs from the tRNA of a prokaryote like *E. coli* and resembles that of eukaryotes. The initiator tRNA of *M. smegmatis* has been sequenced to find out the disposition of these methylated bases<sup>14</sup>. The structure of this tRNA also appears to be unique in that it occupies a place midway between prokaryotes and eukaryotes, as shown by the sequences at positions 1, 54, 57 and 72 of the tRNA.

(8) A significant breakthrough in understanding the genetics of mycobacteria and their drug resistance was achieved by the isolation of a transducing mycobacteriophage for the first time<sup>15</sup>. Amber mutants of this bacteriophage have been isolated using genetic engineering techniques. Studies are under way to map the genes responsible for drug resistance in mycobacteria.

### Selected Publications

1. Ramakrishnan T, Murthy P S & Gopinathan K P, Metabolism of mycobacteria, *Bact Rev*, **36** (1972) 65.



2. Indira M & Ramakrishnan T, Glucose dissimilation by *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub>, *Am Rev Tuberc*, **88** (1963) 509.
3. Ramakrishnan T, Indira M & Maller R K, Evaluation of routes of glucose utilization in virulent and avirulent strains of *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub>, *Biochim biophys Acta*, **59** (1962) 529.
4. Murthy P S, Sirsi M & Ramakrishnan T, Tricarboxylic acid cycle and related enzymes in cell-free extracts of *Mycobacterium tuberculosis*, *Biochem J*, **84** (1962) 263.
5. Gopinathan K P, Sirsi M & Ramakrishnan T, Nicotinamide adenine nucleotides of *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub>, *Biochem J*, **83** (1963) 444.
6. Sriprakash K S & Ramakrishnan T, Properties of isoniazid-resistant mutants of *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub>, *Gen Microbiol*, **60** (1970) 125.
7. Gayatri Devi B, Shaila M S, Gopinathan K P & Ramakrishnan T, The purification and properties of peroxidase in *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub> and its possible role in the mechanism of action of isonicotinic acid hydrazide, *Biochem J*, **149** (1975) 187.
8. Jayaram H N, Ramakrishnan T & Vaidyanathan C S, L-asparaginases from *Mycobacterium tuberculosis* strains H<sub>37</sub>R<sub>a</sub> and H<sub>37</sub>R<sub>v</sub>, *Archs Biochem Biophys*, **126** (1968) 165.
9. Subba Reddy V V, Jayaram H N, Sirsi M & Ramakrishnan T, Inhibitory activity of L-asparaginase from *Mycobacterium tuberculosis* on Yoshida ascites sarcoma in rats, *Archs Biochem Biophys*, **132** (1969) 262.
10. Allaudeen H S & Ramakrishnan T, Biosynthesis of isoleucine and valine in *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub>, *Archs Biochem Biophys*, **125** (1968) 199.
11. Harshey R M & Ramakrishnan T, Purification and properties of DNA-dependent RNA polymerase from *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub>, *Biochim biophys Acta*, **432** (1976) 49.
12. Ramamurthy B, Maller R K, Rao G R, Ramakrishnan T & Bhat M V, N-[2-naphthyl] glycine hydrazide—A potent inhibitor of *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub>, *J Indian Inst Sci*, **60** (1978) 205.
13. Srivastava Rakesh, Gopinathan K P & Ramakrishnan T, DNA methylation in mycobacteria, *J Bact*, **148** (1981) 716.
14. Vani B R, Kuchino Y, Nishimura S & Ramakrishnan T, Nucleotide sequence of initiator tRNA from *Mycobacterium smegmatis*, *Nucl Acs Res*, **12** (1984) 3933.
15. Sundar Raj C V & Ramakrishnan T, Transduction in *Mycobacterium smegmatis*, *Nature, Lond*, **228** (1970) 280.

## V Ramalingaswami

The work of Ramalingaswami relates to pathology of nutritional diseases carried out in India, under Indian conditions, extending over a period of 35 years. Its basic feature is to understand better the mechanisms underlying the development of abnormalities in nutritional disorders through experimental models, including the use of non-human primates and then carry the findings to the clinical and field settings for application wherever feasible.

### **(1) Protein Calorie Malnutrition (Kwashiorkor, Marasmus and Intermediate Forms)**

Ramalingaswami and his colleagues described the syndrome of protein calorie malnutrition in India in 1947 which helped in its recognition subsequently as a major nutritional disorder of infants and growing children of India and other developing countries. Along with M G Deo, he reproduced in 1960 all the essential features of kwashiorkor in rhesus monkeys and described the body reaction to protein depletion in terms of biochemical and morphological sequences. They showed that the fundamental lesion in protein deficiency is one of prolongation of cell generation cycle. This work was summarized in the 15th Annual Ciba Foundation Lecture delivered in London in 1963<sup>1</sup>.

An offshoot of this experimental work on nutrition as an important modulator of immune response provided the basis for the concept of synergism between malnutrition and infection<sup>2</sup>. In one of his key papers<sup>3</sup>, he summarized the clinical and public health ramifications of the experimental work on protein malnutrition.

This series of studies on experimental protein malnutrition threw light on the regulatory mechanisms of cellular proliferation and the relationship between nutrition and cellular growth and differentiation.

### **(2) Nutritional Liver Injury**

Two significant contributions have been made by Ramalingaswami and his colleagues in the field of nutritional liver injury: (1) The experimental demonstration that the fatty liver of protein depletion is due to the failure of the liver to secrete triglyceride, and (2) protein deficiency, far from causing cirrhosis of the liver, produces conditions unfavourable to the development of both of the main features of cirrhosis, namely, fibre genesis and fibrous tissue proliferation and hepatocellular regeneration. These and other studies on nutrition and liver disease, in a variety of experimental animals, including non-human primates and humans, formed the subject of the Heath Clark lecture

---

Director-General, Indian Council of Medical Research, Ansari Nagar, New Delhi 110 029; Residence : 3, Tuglak Lane, New Delhi-110011.



given by Ramalingaswami at the London School of Hygiene and Tropical Medicine in 1972<sup>4</sup>.

### **(3) Liver Disease in the Tropics**

Along with his clinical colleagues, Ramalingaswami described a new syndrome of portal hypertension which was masquerading until then as tropical splenomegaly and as cirrhosis of the liver and later established its essential pathology as one affecting the intrahepatic branches of the portal vein. This work has helped the profession in recognizing the syndrome as a separate entity in different parts of India and indeed it has now been recognized to occur in several other countries. Its significance lies in the fact that it has a different natural history and a much better prognosis than that of cirrhosis of the liver<sup>5</sup>.

Indian childhood cirrhosis is a unique disease affecting infants and young children and is attended by grave prognosis. Along with N C Nayak, Ramalingaswami established the pathological features of the liver in this condition and characterized them ultrastructurally and histochemically. This work has enabled the working pathologist to make an accurate pathological diagnosis of this condition<sup>6</sup>.

### **(4) Himalayan Endemic Goitre**

Today's estimate of people suffering from endemic goitre in India is of the order of 120 million persons. The bulk of them live in the Sub-Himalayan Goitre belt. The aetiology of this condition was shrouded in mystery until 1955 and no significant measures were in force to control it. By taking modern methods of radio-isotope technology and biochemical analysis to the Himalayan field stations, Ramalingaswami

and his colleagues established by 1961 that the cause of this condition was simply severe environmental deficiency of iodine and that no goitrogens or genetic factors were involved. They further studied in humans and animals the mechanisms of adaptation of the thyroid gland to iodine deprivation and drew attention to the exquisite attempt made by the thyroid in conservation of iodine and in enhanced synthesis and secretion of triiodothyronine. The patho-physiology of endemic goitre has been described and the pituitary-thyroid axis investigated<sup>7</sup>.

On the basis of this knowledge, Ramalingaswami and his colleagues launched a large-scale field study in collaboration with the local public health authorities in 1956 in the Kangra Valley, demonstrating the beneficial effect of adding small physiological amounts of potassium iodate to common salt in controlling this disease in a population of 100,000 persons. Eighteen years of careful followup study confirmed that goitre has been controlled effectively in Kangra Valley. This work formed the basis of the National Goitre Control Programme now in operation in India and in other countries of South East Asia<sup>8</sup>.

### **(5) Nutritional Anaemia in Women and Children**

Systematic studies in pregnant women in rural and urban areas of Northern India with S K Sood demonstrated high prevalence rate of anaemia and that the anaemia was mainly due to deficiency of iron. Iron supplements during pregnancy have been shown in controlled field studies to exert a marked beneficial effect on the haemoglobin level<sup>9</sup>.

## (6) Medical Education

Moulding Indian medical education traditionally based on the European models into patterns suitable for India and other developing countries is a part of Ramalingaswami's interests. This work has exerted wide influence both in India and abroad over the movement for improved teaching in medical schools, with special reference to community orientation of medical education<sup>10</sup>.

## (7) Science and Technology and Health Improvement

In recent years, Ramalingaswami has been engaged in planning research strategies at both country and global levels in the application of scientific knowledge already available to third world countries through Health Systems Research<sup>11</sup>.

Under his chairmanship, a joint panel of the Indian Council of Medical Research and the Indian Council of Social Science Research prepared a monograph on "*Health for All : An Alternative Strategy*" in 1981, which is considered a landmark in health development in the country, the essence of which is reflected in the National Health Policy and in the Seventh Five Year Plan strategies.

## Selected Publications

1. Ramalingaswami V, *Perspectives in protein malnutrition*, Substance of the Annual Ciba Foundation lecture delivered in London, 7 October 1963; *Nature*, **201** (1964) 546-51.
2. Bhuyan U N & Ramalingaswami V, Lymphopoiesis in protein deficiency: Stathmokinetic and tritiated thymidine uptake studies of the mesenteric lymphnode of the guinea-pig, *Am J Path*, **75** (1974) 315-26.
3. Ramalingaswami V, Interface of protein nutrition and medicine in the tropics, *Lancet* (1969) 733-35.
4. Ramalingaswami V, *Lancet* (25 November 1972) (Editorial).
5. Ramalingaswami V, Wig K L & Sama S K, Cirrhosis of the liver in Northern India, *Arch Int Med*, **110** (1962) 350-58.
6. Nayak N C & Ramalingaswami V, Pathologic morphology of the liver in *The Liver and biliary system in infants and children*, edited by R K Chandra (Churchill Livingstone, Edinburgh; London and New York) 1979, 124-45.
7. Ramalingaswami V, Endemic goitre in South-East Asia—New clothes on an old body, *Ann Internal Med*, **78** (1973) 277-83.
8. Sooch S S, Deo M G, Karmarkar M G, Kochupillai N, Ramachandran K & Ramalingaswami V, Prevention of endemic goitre with iodized salt, *Bull Wld Hlth Org*, **49** (1973) 307-12.
9. Sood S K, Ramachandran K, Mathur M, Gupta K, Ramalingaswami V, Swarnabai C, Pouniah J, Mathen V I & Baker S J, WHO sponsored collaboration studies on nutritional anaemia in India, *Q J Med (NS)*, **44** (1975) 241-58.
10. Ramalingaswami V, Educating tomorrow's doctors: The role of medical colleges in developing new systems, *Br J med Educ*, **6** (1972) 114-16.
11. Ramalingaswami V, Methods, equipment and techniques for rural health care and their evaluation, *Proc R Soc (B)* (1980) 83-88.



## B Ramamurthi

The most important accomplishment of Ramamurthi has been the establishment of the speciality of brain surgery, which was till then not existing in the country. This required convincing the public and the profession about the possibilities of brain surgery and also to train them to recognize curable brain lesions. Gradually, the efforts succeeded and neurosurgery became established in the country in many centres. More than 40 neurosurgeons were trained during this period.

The scientific contributions have been both in clinical and basic sciences. It was shown that the operation of decompressive laminectomy had a useful place in certain types of paralysis arising from spinal cord injury, caused by fractures of the spine at the dorsolumbar level.

The entity of chronic spinal extradural inflammation of non-tuberculous origin was recognized and a suitable treatment proposed. Clinical entities like cysticercosis, bleeding in pituitary tumours and arachnoiditis of the spinal cord were described and evaluated. The pathogenesis of tuberculoma of the brain was postulated. It was shown that persons with very low resistance to infection developed tuberculous meningitis, whereas those with a mild degree of resistance developed tuberculomas of the brain. A cortical type of infiltrating tuberculoma

was described. The most useful policy in the treatment of tuberculomas was outlined.

The value of the technique of hypothermia in the operative treatment of massive brain lesions was confirmed. Huge vascular meningiomas and arteriovenous malformations of the brain were tackled under hypothermia.

It was observed that subarachnoid bleeding caused by aneurysms of the cerebral blood vessel was not as common in India as was reported in the Western world. It was shown that atherosclerosis of cerebral blood vessels was uncommon in India, probably due to dietary factors and this could explain the low incidence, apart from the factor of missed diagnosis.

Extensive work was conducted in the field of stereotactic surgery and useful observations were made on the anatomy and physiology of the deeper structures of the brain. The value of placing small discrete lesions in the various deep areas of the brain in conditions of epilepsy, abnormal movements and aggressive behaviour disorders was established. During these studies, the role of deep structures in the brain in gastric acid secretion was identified. The postero-medial hypothalamus and the amygdala, structures in the limbic (emotional) system, have control over gastric acid

secretion, stimulation causing increase in acid secretion and destructive lesions a fall.

The value of accurate stereotactic surgical procedures in the treatment of specific mental illnesses had been outlined. Cingulum lesions in obsessive neurosis and basofrontal lesions in severe depression have been found most useful.

In the field of head injury, many new facets were identified in both clinical and epidemiological aspects. It was found that psychological damage occurring in children after head injury was quite different from that in adults. The variations in types of injury encountered in the country were emphasized and treatment patterns evolved, which will suit the conditions all over the country.

Too early neurological signs not observed earlier were described.

In the field of brain tumours, apart from establishing the appropriate techniques of surgery, a clinicopathological analysis was made and the pattern of brain tumour incidence in the country emphasized. The importance of recognizing multiple as well as multiloculated brain abscesses was stressed.

Research was undertaken into the role played by solar magnetic influence on the occurrence of epilepsy in patients. It was proved that geomagnetic storms caused by solar eruptions increased the frequency of epileptic attacks.

The usefulness of nonvolitional biofeedback of brain waves in patients with higher nervous disorders has been established.

## Selected Publications

1. Ramamurthi B, Late laminectomy in traumatic paraplegia, *Indian J Surgery*, **16**(2) (1952) 183-84; *Excerpta medica*, **6** (1953) 1462.
2. Ramamurthi B, Anguli V C & Narasimhan S T, Pituitary apoplexy, *Neurology (India)*, **1**(2) (1954) 60-64.
3. Ramamurthi B, Rajagopalan V & Natarajan M, Hypothermia in massive intracranial tumours, *Neurology (India)*, **9**(1) (1961) 12-19.
4. Ramamurthi B & Varadarajan M G, Diagnosis of tuberculomas of the brain: Clinical and radiological correlation, *J Neurosurg*, **18**(1) (1961) 1-7.
5. Ramamurthi B & Kalyanaraman S, Stereotactic thalamotomy for pain relief, *J R Coll Surg Edinb*, (1966) 46-48; *Excerpta Medica*, **20** (1967) 5379.
6. Ramamurthi B, Stimulation responses in the diencephalon, *Neurology (India)*, **15**(3) (1967) 123-26.
7. Ramamurthi B, Incidence of intracranial aneurysms in India, *J Neurosurg*, **30**(2) (1969) 154-57.
8. Ramamurthi B, Balasubramaniam V, Kalyanaraman S, Arjundas G & Jagannathan K, Stereotaxic ablation of the irritable focus in temporal lobe epilepsy, *Confinia Neurologica*, **32** (1970) 316-21; *Excerpta Medica*, **25** (1972) 860.
9. Ramamurthi B, Two early neurological signs, *Lancet*, **2** (1971) 7728.
10. Ramamurthi B & Ramaswamy V, Psychological damage after head injury in children and adults, *Neurology (India)*, **20** (Suppl 2) (1972) 360-63.
11. Ramamurthi B, Intracranial tumours in India: Incidence and variations, *Int Surg*, **58**(8) (1973) 542-47.
12. Ramamurthi B, Progress in stereotaxic surgery in Madras, A tribute to Dr Spiegel on his 80th Birthday, *Confinia Neurologica*, edited by E A Spiegel, **37** (1975) 384-98.
13. Ramamurthi B, Mascaren M & Valmikinathan K, Role of the amygdala and hypothalamus in the control of gastric secretion in human beings, *Acta Neurochirurgica*, **24** (1977) 187-90.
14. Ramamurthi B & Tandon P N, *Textbook of neurosurgery* (Orient Longman) 1980.
15. Ramamurthi B & Kalyanaraman S, Stereotactic target for epilepsy, *Stereotaxy of the human brain*, edited by George Schaltenbrand and Earl Walker (George Thieme Verlag, New York) 1982.



## Kamal J Ranadive

During my 40 years of work at the laboratories of the Tata Memorial Hospital (1943-52), the Indian Cancer Research Centre, and the Cancer Research Institute, Bombay (1953-80), I have been responsible for laying the foundation of multiaspect basic research on the etiology and mechanism of carcinogenesis at systemic, cell and molecular levels.

### (I) Breast Cancer

In July 1943, under the direction of late Prof. V R Khanolkar, the first experimental biology laboratory was established at the Tata Memorial Hospital, where I studied the spontaneous and chemically induced murine mammary cancer model in inbred strains of mice, elucidating complex interactions of three etiological factors—genetic susceptibility, hormones and milk-borne viral agent (received through mother's milk)—leading to the development of mammary tumour. A series of experiments were conducted to study the morphology of mammary glands and the histology, histochemistry and cytology of integrate endocrine system (ovary, adrenal, pituitary, etc.) from puberty to death, in a large number of mice of five inbred American strains. The data were published in international journals of repute confirming the pathways of action of the three etiological factors, establishing their interaction and relative

importance in mammary carcinogenesis. Hormonal imbalance, particularly hyperestrinism, was proved to be of utmost importance synergetically with milk-borne virus to act on genetically susceptible soil. Two new inbred strains of mice developed by us—ICRC mouse and L(P)—by 1960-62 turned out to be most valuable addition to world's breast cancer models, since ICRC mouse is highly susceptible to both breast cancer and leukemia and was later used a great deal to study viral interference and viral relationship between MTV and MuLV. Multiaspect experimental studies at systemic and cell molecular levels (viral studies) were reported in 46 papers and theses.

### (II) Environmental Cancers Specific to India

The etiology of three of the most common environmental cancers of India was confirmed experimentally.

(1) *Kangri cancer of Kashmir*: It has been possible to prove that this skin cancer on the lower abdominal skin and thighs of Kashmiris using Kangri, a basket with live coal to keep them warm in bitter cold, is caused by smoke of chinar leaves burnt in the kangri and continuous heat. A special strain of mouse, C17, was developed for testing the weak trace of a

carcinogen on its sensitive skin. No other country in the world has such a special strain of mouse to test a weak trace of carcinogen.

(2) *Tobacco chewing and oral cancer* : Forty per cent of the total cancer cases in men in India are of oral cancer. Long-term elaborate studies were carried out during 1954-77, to test the possible carcinogenicity of (i) tobacco, (ii) betel nut, (iii) lime, (iv) betel leaf, etc. Work on chemical fractionation of each ingredient and testing of the carcinogenicity of the active principles of the total extract and their various fractions, etc. on oral mucosa by painting for each ingredient and various combinations of ingredients ran over almost 20 years. Part of this work was financed by the Indian Council of Medical Research. Finally, from mouse skin, subcutis and hamster cheek pouch experiments, it was repeatedly confirmed that by itself tobacco has a weak trace of carcinogenic principle, and the water extract of betel nut, particularly its polyphenolic fraction containing tannins, is highly carcinogenic. Most dangerous is the combination of tobacco, lime and betel nut. About 25 papers resulted from this work.

This finding was also confirmed through short-term *in vitro* organ culture experiments using new-born rat oral mucosa.

(3) *Edible oils and carcinogenicity* : Two types of problems are discovered with reference to edible oils consumed daily in the Indian food. The first of these relates to the use of solvent extracted oils. If cheap nonfood grade solvent N-hexane with a wide boiling range is used for solvent extraction, some residual solvent remains in the oil and causes cancer. The

second problem is caused by argemone contamination in mustard oil which was proved to be highly hazardous.

### (III) Cell, Tissue and Organ Culture Studies

I had the privilege of setting up the first tissue culture laboratory in India in 1953 at the newly inaugurated Indian Cancer Research Centre, Bombay, set up by the Health Ministry. During my postdoctoral training in 1950-51, I spent one year under Rockefeller Foundation Fellowship to pick up tissue culture technology for *in vitro* cell and molecular level studies on cancer cells. At our new tissue culture laboratory, I spent the first two years in standardizing the basic fastidious technology to be able to grow under continuous cultivation cancer cell lines for a variety of experiments and we succeeded remarkably. The world's very first and the best human sarcoma cell strains have been kept in continuous cultivation since 1957. We also started our own experimental model mouse sarcoma cell line MFS8 and its several single cell clones the same year. This stock of cells has been in continuous use for experiments in carcinogenesis, chemotherapy and multiaspect cell biological studies, at our laboratories and in many other laboratories in India. Spontaneous and chemical carcinogenesis-cell transformation studies have been carried out on several human sarcoma cells, mouse sarcoma cell clones and human diploid cell strains. Besides our own stock of cells, we supplied commercial cell lines to all the tissue culture laboratories in India as part of the maintenance and supply service of ICMR for 12 years. We were the first to start time-lapse cinematography of living cells and have photorecorded many unique



phenomena, e.g. phagocytosis (cannibalism in culture), cell fusion, nucleolar extrusions, Feulgen positive material in cytoplasm, cell transformations, etc. Our films are used by many as educational material to study phenomena like cell division, cell transformation, etc.

Today, living cell material and modern techniques like hybridoma are used in my laboratory for immunobiological studies.

#### **(IV) Human Leprosy**

Human embryonic spinal ganglia cultures (SPG) were used for the isolation and cultivation of acid-fast micro-organisms from human leprosy nodules. The ICRC bacillus isolated from lepromatous leprosy nodules in monolayer cultures of SPG (human) cell in 1957 was later transferred to a modified fluid medium of cancer stock cells. ICRC bacillus has been repeatedly isolated and kept in continuous cultivation successfully. Now transferred to almost complete synthetic medium, it is a precious candidate for the preparation of vaccine for leprosy.

#### **(V) Training of Young Scientists**

The multiaspect biological work at animal, cell and molecular levels ended officially in December 1977 on my retirement as Head, Biology Division, Cancer Research Institute, but it was continued till December 1979 in my capacity as Emeritus Scientist so as to complete some laboratory projects of ICMR. A well trained cohesive group of young scientists is carrying the torch further. There were 45 scientists, most of whom had done their one or both post-graduate degrees with me as Bombay University teacher and all were sent abroad for specific topical post-doctoral

training. They are now heading various groups.

#### **(VI) Cancer of Ignorance of Facts of Life**

In 1979-80, I switched over to field studies on nutrition and cancer in tribal population. The results of our laboratory studies on nutrition and cancer prompted me to undertake these studies, since the laboratory work indicated that breast cancer in mice can be inhibited by feeding control diet of low calories, low fat and just enough proteins. Out of necessity, tribals eat exactly the same kind of food—low calorie, low fat, etc. The question was whether this population, though prone to oral cancer and cervix cancer, has protection from breast cancer because of their dietary habits! This work is going on as a sideline, but the main emphasis today is on helping to eradicate cancer of ignorance about facts of life in these downtrodden people. With a high infant mortality rate because of infectious diseases and faulty nutrition, thousands are being born and die like flies.

Today, I am busy with my diet and health surveys in these tribal areas of Akola Taluka in six villages and have recently started a health education programme for tribal women—an integrated approach to tribal welfare. Last four years' work has yielded highly satisfactory results and there is hope we might be able to help these people to live a better life following the philosophy of "Health for all by 2000 AD".

#### **Selected Publications**

##### **(I) Breast Cancer**

1. Ranadive Kamal J & Khanolkar V R, The effect of foster-nursing on the morphology of the

mammary glands in mice, *J Path Bact*, **59** (1947) 593-603.

2. Ranadive Kamal J & Hakim S A, Biological study of strain L(P) and its response to 20-MCA treatment, *Br J Cancer*, **12** (1958) 44.
3. Ranadive Kamal J, Hakim S A & Kharkar K R, Chemical induction of mammary cancer in pseudopregnancy, *Br J Cancer*, **14** (1960) 508.
4. Karande K A & Ranadive Kamal J, Influence of hormones and chemical carcinogen on murine leukemia, *Br J Cancer*, **28** (1973) 299.
5. Hiraki S, Ranadive Kamal J & Dmochowski L, An electron microscopic study of spontaneous and experimentally induced leukaemia in ICRC mice, *Cancer Res*, **34** (1974) 474-83.
6. Karande K A, Joshi B J, Talageri V R, Dumaswala R U & Ranadive Kamal J, Characterisation of mammary tumour virus of strain ICRC mouse, *Eur J Cancer*, **14** (1978) 251-61.

## (II) Environmental Carcinogens

7. Gothoskar S V & Ranadive Kamal J, Experimental studies on the aetiology of 'Kangri' cancer, *Br J Cancer*, **20** (1966) 751.
8. Ranadive Kamal J, Gothoskar S V, Rao A R, Tezabwalla B U & Ambaye R Y, Experimental studies on betel nut and tobacco carcinogenicity, *Int J Cancer*, **17** (1976) 469-76.
9. Ranadive Kamal J, Ranadive S N, Shivaspurkar N M & Gothoskar S V, Betel quid chewing and oral cancer: Experimental studies on hamsters, *Int J Cancer*, **24** (1979) 835-43.
10. Ranadive Kamal J & Gothoskar S V, Testing carcinogenicity of edible oils: Part I (Peanut oil) and Part II (Mustard oil), *Indian J med res*, **53** (1965) 975-79; 980-83.

## (III) 'In vitro' Studies (Cell Biology)

11. Ranadive Kamal J, Ganguly B & Mashelkar B N, Cytological study with reference to malignancy

on clonal cell lines of a mouse fibrosarcoma, *Int J Cancer*, **3** (1968) 39-50.

12. Bhisey A N & Ranadive Kamal J, Cinematographic and cytochemical studies on phagocytosis in mouse fibrosarcoma cell line MFS<sub>8</sub> cultivated 'in vitro', *Exp Cell Res*, **44** (1966) 139-49.
13. Ranadive Kamal J, Wagh U V, Vernekar S V, Bhisey A N & Bose S, Carcinogenesis in tissue culture: (a) Spontaneous and (b) Chemically induced, *Proc International Conference of Tissue Culture*, University of Tokyo, 1968.
14. Ranadive Kamal J & Gangal S G, Phenotypic and karyotypic alterations in mammalian cells in tissue culture, *Proc Symposium on the Characterisation & Uses of Human Diploid Cell Strains*, Yugoslavia, Opatija, 1963.
15. Ranadive Kamal J & Bhide Sumati V, Tissue interactions between normal and malignant cells, *Proc Henry Ford Hospital International Symposium on Biological Interactions in Normal and Neoplastic Growth*, 1962.
16. Chapekar T N, Nayak G V & Ranadive Kamal J, Studies on the functional activity of organotypically cultured mouse ovary, *J Embryol exp Morph*, **15** (1966) 133-41.

## (IV) Leprosy

17. Ranadive Kamal J, Nerurkar R V & Khanolkar V R, 'In vitro' studies on human leprosy, *Indian J med Sci*, **12**(1958) 791-96.
18. Bapat C V, Ranadive Kamal J & Khanolkar V R, Growth characteristics of an acid fast mycobacterium isolated from human lepromatous leprosy, *Int J Lepr*, **29** (1961) 329-42.
19. Ranadive Kamal J, Bapat C V & Modak M S, Conditioned fluid of mammalian stock cells 'in vitro' as nutritive medium for acid fast ICRC bacillus isolated from lepromatous leprosy, *Proc International Symposium on Cell Nutrition*, Tokyo, Japan, 1977.



## A P Ray

A brief account of the major research contributions of Ray is given below.

### (1) Parasitology

Working at the Malaria Institute of India (now National Institute of Communicable Diseases) and in collaboration with Medical Research Centre, Kuala Lumpur, Malaysia, Ray and his colleagues were able to isolate a species of simian malaria (*P. knowlesi*) in *M. irus* monkey of Malaysia. This strain proved to be extremely virulent in rhesus monkey of India and thus served as an excellent experimental model for screening of antimalarial drugs and other studies. The model simulated *P. falciparum* (malignant tertian malaria) in man.

This enabled systematic studies on screening of many potential antimalarial drugs to be undertaken. This strain was later utilized by other laboratories in India.

Two new avian malaria parasites were identified in partridges in India. While working in Australia, Ray was responsible for the detection of a plasmodium in wood ducks.

### (2) Pathology

Some of the more significant observations made by Ray are related to the pathogenesis of liver injuries associated with severe acute malaria

infection and those caused in repeated chronic infection in subjects living under low protein diet.

The work was undertaken in simian plasmodia and rhesus monkey (*M. mulatta mulatta*) models, such as (i) *P. knowlesi* in rhesus monkeys simulating acute infection with *P. falciparum* in man, and (ii) *P. cynomolgi* in rhesus, and with repeated challenges under normal conditions and under various dietary deficiencies, simulating chronic *P. vivax* infection in man in communities living under conditions of varied level of protein malnutrition.

Experimental studies with simian model demonstrated liver damage in *P. knowlesi* infection, the nature and degree depending on the level of infection. In severe cases, there is centrilobular necrosis, culminating in extensive damage to the organ. The immediate factors responsible are slowing of blood flow and later stasis in the central hepatic vein caused by the action of a sphincter mechanism in the hepatic venous tree. It has also been demonstrated that bilateral sympathectomy splanchnic prevented such liver damage. Similar results were obtained after bilateral hypothalamic electrolytic lesions produced stereotactically in the antero-medial portion of median eminence of hypothalamus. The same is

achieved by combating anoxaemia through transfusion of blood or packed cells, but not of plasma. Earlier studies by other workers had indicated that in severe malaria infection there is excessive adrenal secretion possibly by stimulation of some part of thalamus.

Against the above background, the sequence of events was supervised as severe anoxaemia caused by malaria, stimulating the median eminence of hypothalamus, causing excessive adrenal secretion, which, in turn, activates the sympathetic nervous system setting the sphincter mechanism into action in the central hepatic venous tree. This then produces stasis in the central hepatic veins, thus causing anoxia and necrosis of hepatic cells round the central veins. In very advanced cases, the liver lobules are damaged and disrupted seriously.

The entire process is reversible by transfusion of blood, thus saving life. It is now well known that in some advanced cases of falciparum malaria without supportive measures, specific treatment by itself is unlikely to prevent death.

Experimental studies showed repeated infection with *P. cynomolgi* in rhesus monkeys kept under various dietary conditions, including varying grades of protein malnutrition, simulating conditions prevailing in many parts of the country among many human communities.

The observations indicate that pathological changes in liver occur after repeated infection with malaria in subjects kept under various degrees of protein malnutrition. On prolonged exposure to such conditions, the injuries appear to be similar to those noted in cirrhosis of liver. But in this case, the process is often

reversible after adequate protein intake, both of animal (milk) and vegetable (peanut-soyabean milk) origin. Vitamin B complex or any of its components like niacin had no effect.

These observations have a tremendous significance. The economically weaker sections of the community live under conditions of marginal level of protein in the diet. It is also well known that every malaria attack is liable to deplete body proteins. Thus, in case of repeated infections, pathological changes in liver are expected.

The degree of liver damage, as shown in experimental studies, would depend on the number of malaria attacks and the level of protein malnutrition. It was also demonstrated that in most instances, the lesion is reversible when adequate protein of animal or vegetable origin is supplied in the diet.

### (3) Chemotherapy

Against the only drug, quinine, in the past, a variety of synthetic preparations of different chemical groups and varied types and degrees of effectiveness at different phases of plasmodial life cycle in vertebrate and invertebrate hosts, became available from the late forties. The research activities on antimalarials which were responsible for the advent of many new preparations were prompted by the two World Wars. The third stimulus was the emergence of drug resistant malaria foci in different parts of the world from the sixties.

Ray had the opportunity to work in this area in various research centres in USA and UK. Work at the erstwhile Malaric Institute of India, Delhi (now National Institute of Communicable Diseases)



included (i) studies on avian, rodent and simian models with the corresponding plasmodial infections and chemotherapeutic trials, (ii) controlled studies on human subjects under hospital conditions, (iii) field trials and standardization of various treatment schedules, and (iv) systematic studies on the emergence of resistance to drugs in plasmodia.

One of the studies of far reaching significance was the emergence of resistance to 4-aminoquinolines after prolonged exposure.

Assisted by a colleague, Ray was the first to demonstrate during experimental studies that prolonged exposure precipitated the development of resistance in a plasmodium even to the most powerful antimalarial drug like chloroquine of the 4-aminoquinoline series. On further exposure, the level of resistance increased by 26 fold.

The significance of this finding (first reported in *Nature*, 1956) will be evident from the following text from an internationally acknowledged book on chemotherapy and drug resistance (Peters, 1970).

"It was some 10 years after chloroquine appeared before Ray and Sharma (1956) published the first report of resistance to chloroquine in *P. gallinaceum*. Two years later (Ray *et al.* 1958) pointed out an increase by 26 fold resistance in the same plasmodium. But their papers seem to have received little attention. Meanwhile everyone showed their faith in chloroquine in an ever increasing dose in tablets and also in cooking salts under the tranquil illusion that here was an antimalarial towards which the malaria parasite would not become tolerant. Then came in 1961 the first report that *P. falciparum* in

Colombia was not responding to chloroquine. Suddenly the malariologists were shaken out of their complacencies."

Then began the search for alternative drugs. It is still on.

Later, being involved in the field of epidemiology, control/eradication of malaria programme in the country, Ray was responsible for evolving the most practical and scientific treatment schedules mostly under rural conditions, particularly in relation to the baffling problem of prevention of relapses.

In the late sixties and early seventies, while working under WHO, Ray and his associates were able to demonstrate the emergence of resistance in *falciparum* strains to some of the 4-aminoquinolines like amodiaquine in human subjects in the Philippines, with evidence of dissemination of such resistant strains to other parts.

As Director, Malaria Research Unit in Australia, Ray was involved in research on drug resistance and the search for alternative antimalarial drugs.

From the early eighties, as chief-coordinator, Government of India & SIDA/WHO, Ray has been assisting the *P. falciparum* containment project under the National Malaria Eradication Programme in monitoring and dealing with the problem of drug resistance in certain parts of India and demonstrating measures to prevent the dissemination of such strains of *falciparum* malaria to other parts of the country.

Of the more than 150 papers published in national and international scientific journals, about 50 pertain to chemotherapeutic aspects.

Ray has been a member of the WHO Expert Committee Panel on Malaria from 1960.

#### (4) Miscellaneous Activities

Extensive researches were carried out on rhesus monkey (*M. mulatta mulatta*) and simian plasmodia model over several decades at the erstwhile Malaria Institute of India, Delhi. Ray played a significant role in these studies. He recognized quite early that the wild monkeys caught mainly from rural areas are prone to tuberculosis. Detailed investigations by him and his colleagues revealed the nature of infection and the lesions in organs involved which are similar to those found in man. What appears to be most significant is the finding made in collaboration with the Indian Veterinary Research Institute (IVRI), Mukteswar, that the tubercle bacilli are of human type. Subsequent studies proved that the monkeys could be experimentally infected by human tubercle bacilli.

On the basis of these observations, Ray advised the IVRI and other institutions to undertake systematic studies on monkeys, including those of urban areas, specially from localities where the prevalence of the disease in man is high.

#### (5) Control and Eradication of Malaria

For about eight years (1960-67) Ray was the Director, National Malaria Eradication Programme, the largest single health programme in the world. In this capacity, he was totally involved in all aspects of the programme. Having worked from grassroot level in all disciplines of the subject of malariology, he helped in bringing at least one health programme to the door of the people in terms of intervention measures, study of the impact

through epidemiological parameters and developing laboratory services in the field.

During this period (1962) he wrote the monograph "The National Malaria Eradication Programme (India), Technical Directives and Administrative Guidance", which has become the *vade mecum* of all malariologists. It still remains the most sought after document in the programme in India and abroad.

By 1966/1967, malaria was eradicated from more than half the population area in the community.

Ray has been a member of the WHO Expert Committee Panel on Malaria since 1960. During 1960-67, he was assigned by the WHO in different capacities the responsibility of assessing and advising malaria eradication programmes in a number of countries. Subsequently, as a senior malaria adviser in the Western Pacific Region of WHO, he was closely associated with programmes in the countries of that region.

#### (6) Epidemiological Aspect (Including Entomology)

Ray has been associated with the field of epidemiology of malaria for about 45 years, and his contributions have been many. Under the campaign, he was the principal architect in developing and sustaining a system of epidemiological surveillance involving a series of disciplines and dynamics. One of the most essential components is the system of domiciliary service in the entire country established from 1961, ensuring total geographical coverage with a well defined regular periodicity based on technical and administrative needs considerations. The service is designed to search for malaria cases through periodical contacts to



diagnose, through laboratory services, ensure adequate treatment and define remedial measures as guided through epidemiological investigations.

The system has now come to stay in this country and is providing a platform for extending other health activities to the people under the multipurpose workers scheme.

In a recent paper (June 1984), Ray and his colleague have elaborated on the theme of epidemiological surveillance as a tool for measurement of malaria and its control. Earlier, a paper was published on evaluation through socio-economic impact.

### Selected Publications

1. Jaswant Singh, Ray A P & Nair C P, Transmission experiments with *P. knowlesi*: Parts I and II, *Indian J Malariol*, **3** (1949) 145-48; **4** (1950) 317-27.
2. Taylor D J, Greenberg J, Ray A P & Josephson E S, Studies on *P. gallinaceum* in vitro, *J infect Dis*, **88** (1951) 158-62.
3. Ray A P & Sharma G K, Experimental studies on liver injuries in simian malaria (*P. knowlesi*), Parts I, II and III, *Indian J med Res*, **46** (1958) 359-67; 367-76; *Bull Soc Indian J Mal Mosq Dis*, **6** (1958) 173-75.
4. Ray A P, Role of blood transfusion in liver injuries due to severe *P. knowlesi* in rhesus monkeys, *Bull natn Soc Ind Mosq Borne Dis*, **2** (1954) 213-14.
5. Nair C P & Ray A P, Observation on the incidence and type of tuberculosis in rhesus monkeys, *Indian J Malariol*, **9** (1955) 185-86.
6. Nair C P & Ray A P, Tuberculin test in diagnosis of tuberculosis in rhesus monkeys, *Indian J Tuberc*, **1**(2) (1955) 1-4.
7. Ray A P & Jaswant Singh, Studies on some aspects of nutritional status of *M. mulatta mulatta* (rhesus monkeys) in nature and captivity, *Lab Anim Bur Coll Pap* (Medical Research Council, London) 1935.
8. Ray A P, Experimental studies on liver injury in malaria effect of malnutrition in monkeys. Parts I, II and III, *Indian J Path Bact*, **2** (1959) 293-303; 304-9; **3** (1960) 1-16.
9. Taylor Jane, Greenberg J, Josephson E S & Ray A P, A metabolic intermediate of primaquine from chicken (blood and tissues), *Exp Biol Med*, **76** (1951) 700-3.
10. Jaswant Singh, Ray A P, Basu P C & Nair C P, Acquired resistance to proguanil in *P. knowlesi*, *Trans R Soc Trop Med Hyg*, **46** (1952) 639-49.
11. Ray A P & Sharma G K, Acquired resistance in chloroquine diphosphate in p.g. *Ilinaceum* in chicks, *Nature Lond*, **178** (1980) 1291.
12. Shute G T, Ray A P & Sangalang R, Preliminary studies on a Philippine strain of *P. falciparum* resistant to amodiaquine (4-aminoquinolines), *J trop Med Hyg, Lond*, **75** (1972) 125-33.
13. Ray A P, Parkinson A D & Black R H, Experimental studies on the effect of proguanil and dapsone against chloroquine resistant *P. berghei* (ANKA) in white mice, *Ann trop Med Parasit, Lond*, (1979).
14. Ray A P, The different facets of drug resistant *Plasmodium falciparum* malaria, *Proc Conference on malaria*, Commonwealth Institute of Health, Sydney (Australian Govt Printing Press, Canberra) 1984, 48-54.
15. Ray A P, The problem of dissemination of drug resistant falciparum malaria through population movement, *Proc workshop on Population Movement and Impact on Tropical Disease Transmission*, Paradeniya, Sri Lanka (World Bank, WHO, UNDP) 1984, 117-28.
16. Ray A P, Epidemiological surveillance in malaria eradication campaigns (1964), *Proc Seventh International congress in Tropical Disease and malaria*, Vol 5, Rio de Janerio, Brazil, 1963, 152-58.
17. Ray A P, Some epidemiological aspects in malaria eradication programme, *Bull Indian Soc Malaria Dis*, **3** (1966) 165-84.
18. Ray A P & Beljaev A E, Epidemiological surveillance: A tool for assessment of malaria and its control, *J Communic Dis*, **16** (1984) 197-207.
19. Ray A P, National Malaria Eradication Programme (India), Technical Directives and Administrative guidance, *Bull Malaria Soc India Mosq Dis*, **11** (1963) 59-191.

## C R R M Reddy

The major research contributions of Reddy are discussed below in brief.

### (1) Carcinoma of Hard Palate

Reverse smoking is a common habit in the districts of Visakhapatnam and Srikakulam of Andhra Pradesh. About 25% of males and 50% of females above 20 years smoke reverse. In a survey, 70-80% of them showed stomatitis nicotina, a popular umbilicated lesion of the glandular zone of the hard palate. Cigarette and bidi smoking is not a common habit here.

When whole papules of stomatitis nicotina were biopsied and sectioned serially, mild to severe atypia to microinvasive carcinoma in the squamous metaplastic lining of the ducts draining the palatine glands was found. Almost one-third of reverse smokers showed atypia and less than 2%, microinvasive carcinoma. Severe grades of atypia were seen in the reverse smokers compared to other types of smokers. Microinvasive carcinoma and carcinoma of the hard palate were seen along with stomatitis nicotina.

In the field survey it was found that women with a long history of reverse smoking had regressing stomatitis nicotina. The palate was thickened, whitish or pigmented, with flattened papules and small depressions. Their biopsy revealed hyper-orthokeratosis of the keratinized

epithelium in between the ducts, along with squamous metaplasia of the ductal epithelium. Metaplastic epithelium keratinizes, partially obstructs the ducts and causes elongation and tortuosity of the ducts, papilliferous projections, etc. In these cases, partial atrophy of the glands occurs. If the keratinized closure of the ducts is early, the whole gland atrophies.

When the mean ages of people showing mild atypia, moderate to severe atypia, microinvasive carcinoma, squamous cell carcinoma and hyperorthokeratotic changes were calculated, it was found that atypia occurred earlier, but an interesting finding is that hyperorthokeratosis and cancer occurred at the same age. The reaction of an individual to reverse smoking appears to be keratinization of surface epithelium and also the ducts primarily, but atypia occurs only in a few cases.

Thirty reverse smokers with palatal ulcers were followed up in three villages over a period of three years and it was found that some of them did progress to carcinoma.

The commonest oral cancer seen at Visakhapatnam is hard palate cancer. World's highest incidence of hard palate cancer is seen here. Smoking chuttas is common, but reverse smoking of chuttas is predominant. Chewing is uncommon.



Matching of oral cancer cases against controls and calculation of relative risk according to the Mantel-Haenszel formula revealed that reverse smoking female runs 132 times greater risk compared to a non-smoker. This method of smoking was of no significance to other sites. The ordinary method of smoking is also not of much significance.

When risk was calculated for single and multiple habits, it was again seen that reverse smoking is the most important risk factor to be reckoned with and not any other single or combined habit. Further, if a reverse smoker changes over to ordinary smoking habit, there could be a 80% reduction in the risk of developing palatal cancer.

Macroscopic, microscopic and cytological examination revealed the common occurrence of microinvasive, plaque like fungating, verrucous and perforating. All were squamous cell cancers of low grade localized to the posterior one-half of hard palate.

## **(2) Carcinoma of Penis**

From the available published literature on the frequency with which cancer of penis is seen in various geographical regions of the world it was found that at Visakhapatnam this cancer is quite frequent. In India also, it is most frequent at Visakhapatnam compared to other areas for which published information is available. It forms 12.5% of all male cancers and 5.5% of all cancers.

In all the normal penises studied not even a single gland opening into the preputial sac was seen. In the literature, there is lack of consensus regarding the presence or absence of preputial glands. Smegma is considered at least by some as

a secretion from glands. Absence of the glands could mean that smegma is formed by the exfoliation of the opposing cornified squamous epithelial cell layers of the preputial sac.

To judge the veracity of the statement that cancer cervix might be due to smegma from the uncircumcized husband, the preputial skin of husbands of cancer cervix patients was studied. Abnormalities in the surface epithelium were looked for thinking that smegma which might have caused cancer in cervix could at least have caused some atypical changes in the native epithelium, where the smegma was primarily present. But for mild atypia in a phimotic person there was no other change. The cervixes of the wives of cancer penis patients were examined macroscopically and microscopically to see whether there was any change in the cervix, but drew a blank. From control studies on cancer of penis it was found that phimosis was much more important than circumcision. The penile hygiene, circumcision status and the number of phimotics in a large group of males attending the outpatient department were studied. Phimosis was found to be a very important factor to reckon with.

Preputial skins of circumcized patients were obtained and also the reason why circumcision was resorted to was ascertained. In 42 preputial skins available for this study, 18 had phimosis and 9 of these had atypia (5 mild and 4 moderate). This is an interesting finding. Phimotics do not have a chance of cleaning themselves properly. Moreover, there is every likelihood of urine mixing up with smegma every time the person passes urine. Whether this could give rise to changes in smegma and make it carcinogenic in some is receiving attention.

### (3) Epidemiology of Hydatid Disease at Kurnool

At Kurnool, hydatid disease was found to be quite common; every year more than 10 cases were seen in the Department of Pathology, Medical College, Kurnool. Of the 527 cases of hydatid disease reported in India during 1933-1967; 239 cases were from Kurnool, Guntur, Madras and Vellore; of these, 70 were from Kurnool. Of the 160 casoni tests done over two years, 83 were positive. Among 33 cases operated upon, 25 (75%) showed the cyst and in the other 8 (25%) there was no hydatid cyst. Out of these 8 patients, 6 had disseminated malignancy. A survey was carried out in a nearby village where almost all the people had sheep and rough woollen blanket making was a cottage industry. In a group of 1329 people, excluding the below 5 year age group, we could do the casoni test in 760 people (57.19%); of these, 167 had a positive reaction (21.98%). This showed wide prevalence of the disease in the area.

All the 150 dogs obtained from the streets of Kurnool town when autopsied showed the presence of adult echinococcus worms. This is much more than that reported from anywhere else in the country, but was at par with that reported from other endemic areas of the world. Over a two month period, all the animals slaughtered in the local slaughterhouse were examined for hydatid cysts. 7.7% of pigs, 16.96% of goats, 21.75% of sheep and 60.94% of cattle showed the infection. Since the number of sheep slaughtered was more, the importance of sheep as a source of infection for the dog is important. The proportion of domesticated animals showing hydatid infection is much higher than what is

recorded in the country and almost equal to what is recorded from endemic foci of hydatid disease in the world.

Examination of cysts showed 64.6% of cysts of sheep, 66.7% of buffalo, 54.5% of goats, 41.7% of cows, 31.5% of oxen and 28.5% of pigs to be fertile. The cysts were seen more often in the lungs of the animals and the lung cysts were more fertile than cysts in other organs. Since the ratio of the number of sheep and buffaloes cut in the slaughterhouse is about 150 : 1, the importance of the sheep-dog cycle is evident.

Prospective and retrospective development of material from the fertile cysts (sheep, cow, buffalo, goat, pig and human) was studied in dogs, cats, rats, mice, guineapigs and rabbits. The studies indicated the adult worm to be similar to the worm described from New Zealand, possibly introduced into that country by the European colonists. In India also the hydatid infection could have been introduced or reintroduced by European colonists.

### (4) Guineaworm Infection at Kurnool

Dracontiasis in one of the most easily preventable infections, yet it is common in parts of India. About 10,000 inhabitants of four villages in Kurnool district were interviewed in 1967; prevalence of guineaworm infection was found to vary from 11 to 54% of the inhabitants. The prevalence increased with the proportion of the people using step wells. More than one worm at a time was seen in 40% of the infected persons; more than one infection in a year was seen in 60% of them and infections occurred in more than one year in 62% of them. Reinfections were common and many people suffered for



long periods. Survey of the water sources in the villages in the district showed that 500 out of the 1486 villages had step wells; thus, a population of 0.5 million is at risk.

*Dracunculus medinensis* can cause three types of lesions in human beings. It can cause a bleb through which the worm can come out; it can get calcified; or it can form a sterile abscess called guineaworm abscess. All the three types of the above lesions are caused by a mature gravid female. The blebs are present mostly in the lower limbs.

Analysis of about 10,000 X-ray examinations done in the local hospital revealed 4.6% of them to have calcified guineaworms. The pelvis and abdomen are the commonest sites where these are present. These are seen in the older age groups as opposed to the acute infections which are seen in the younger age groups. The calcified worms as a rule are symptom-free. In some of the persons (29%), the X-ray examinations reveal a calcified worm, but the persons have denied having had an acute infection. Why and how a mature gravid female without coming out or forming an abscess gets calcified remains to be worked out.

The least common of the manifestations is the true guineaworm abscess which usually occurs in the scrotum. This is a chemical abscess caused by the contents of the gravid female worm. One other special site where this can occur is the knee joint, where the worm can cause an acute synovitis. Opening of the joint and evacuation of the contents usually gives back normal joint to the person.

All persons in a family drinking water from the same pot (the water having been brought from a step well) do not get the infection. Hyperchlorhydria was one of the

factors which was thought to prevent infection. Gastric acid studies showed that guineaworm infection can occur irrespective of whether the person has achlorhydria hypochlorhydria or hyperchlorhydria. Presence or absence of bile also did not have any effect.

The worm moves in the subcutaneous tissues and the infective larvae have to penetrate the wall of the gastrointestinal tract to continue further development. Proteolytic activity could be demonstrated against casein gelatin hemoglobin in the secretions of the infective guineaworm larvae. The activity could not be demonstrated or was present only negligibly in the secretions of the noninfective larvae. Proactivator of the plasminogen also could be demonstrated in the infective guineaworm larvae. The gravid female in its contents has proactivator of the plasminogen. These enzyme activities could be of use for the infective larvae or the worm for their migration.

In a village where everyone has no other option except to drink step well water, 54% of the persons were found to have history of active infection and 29% of the people had occult infection in the form of calcified guineaworm. Thus, 83% get infected one way or other and only 17% of the people escape occult or manifest guineaworm infection when every one drinks step well water.

## Selected Publications

### Carcinoma of Palate

1. Reddy C R R M, Kameswari V R, Ramulu C & Reddy P G, Histopathological study of *Stomatitis nicotina*, *Br J Cancer*, **25** (1971) 403.
2. Reddy C R R M, Raju M V S, Ramulu C & Reddy P G, Changes in the ducts of the glands of

the hard palate in reverse smokers, *Cancer*, **30** (1972) 231.

3. Reddy C R R M, Jajkumari K & Ramulu C, Regression of *Stomatitis nicotina* in persons with a long-standing habit of reverse smoking, *Oral Surg, Oral Med & Oral Path*, **38** (1974) 570.
4. Reddy C R R M, Rajkumari K & Kameswari V R, Pathology of carcinoma of hard palate, *Path Microbiol*, **41** (1974) 118.
5. Reddy C R R M, Carcinoma of hard palate in India in relation to reverse smoking of chuttas, *J natn Cancer Inst*, **53** (1974) 615.
6. Reddy C R R M, Hypothesis on the origin of carcinoma of hard palate, *Oncology*, **30** (1974) 134.

#### Carcinoma of Penis

7. Reddy C R R M, Gopal Rao T, Venkatarathnam M D, Kameswari V R, Sasiprabha R & Raghaviah N V, A study of 80 patients with penile carcinoma combined with cervical biopsy study of their wives, *Indian Surg*, **62** (1977) 549.
8. Reddy C R R M, Devendranath V & Pratap S, Carcinoma of penis—Role of phimosis, *Urology*, **24** (1984) 85.

#### Hydatid Disease

9. Reddy C R R M, Narasiah I L, Parvathi G & Rao M S, Epidemiology of hydatid disease in Kurnool, *Indian J med Res*, **56** (1968) 1205.
10. Reddy C R R M & Suvarna Kumari G, Biology of *Echinococcus granulosus* as it occurs in South India, *Acta Tropica*, **28** (1971) 311.

#### Guineaworm Infection

11. Reddy C R R M & Siva Ramappa M, Guinea-worm arthritis of knee joint, *Br med J*, **1** (1968) 155.
12. Reddy C R R M, Sivaprasad M D, Parvathi G & Chari P S, *Ann trop Med Parasit*, **62** (1968) 399.
13. Reddy C R R M, Narasiah I L & Parvathi G, Epidemiological studies on guineaworm infection, *Bull Wld Hlth Org*, **40** (1969) 521.

#### Other Topics

14. Reddy C R R M, Suvarna Kumari G, Devi C S & Reddy C N, Pathology of scorpion venom poisoning, *J trop Med*, **75** (1972) 98.
15. Reddy C R R M, Ramachandra Rao N, Ramakrishna Reddy & Anees A M, Pathology of pulsless disease, *Path Microbiol*, **34** (1969) 10.
16. Reddy C R R M, Parvathi G & Ramachandra Rao N, Pathology of cardiomyopathy in South India, *Br Heart J*, **32** (1970) 226.



## B B Roy

The work of Roy has been related to stress research in mammals and submammalian vertebrates, directed mainly at the study of changes in pituitary-adrenal axis and the changes and functional alterations in the hypothalamus. Stress response in invertebrates too was studied.

In 1951, Roy obtained his MS degree of Calcutta University for his thesis entitled "Prognostic evaluation of acute intestinal obstruction cases with special reference to adrenocortical changes and potassium metabolism".

Apart from clinical examinations, the work covered different biochemical changes in the patients, which had some bearing on the changes in different constituents like serum sodium, potassium and blood chloride and urinary neutral 17-ketosteroids related to physiological alterations in the endocrine glands. Histopathological examinations of the adrenal glands in some cases after death revealed various changes, the significance of which included lipid storage phase, accessory bodies coming into play, regeneration from capsule, distorted zonal arrangement, capsular thickening and interstitial fibrosis, infarction, capillary thrombosis, haemorrhage and inflammatory changes. Degenerative changes in the fascicular zone were noted in cases of

peritonitis. In very acute cases, the precortin phase could not turn over to the cortin phase and big cells in the outer fascicular zone were loaded with lipids in the storage phase. The reticular zone was very much reduced. Compensatory mechanisms include (1) generalized compensatory hypertrophy of the zona fasciculata, (2) nodular hypertrophy, and (3) accessory bodies coming into play. The cortico-medullary relationship is also altered.

Another major project involved clinical and experimental studies designed objectively to evaluate the role of hypothalamo-pituitary-adrenal axis in burns. In burned patients there is increased urinary excretion of corticosteroids. The adrenals and the pituitary are affected in burns in diverse ways; the changes were described. Potassium is a stimulant to the adrenal cortex and it has got adrenal ascorbic acid depletion activity in dogs and guineapigs. It stimulates ACTH secretion. ACTH had a greater stimulating action on the adrenal cortex of a burned dog than in the normal one. The adrenal venous effluent after increasing potassium in the intracranial circulation in dogs had a better action on the adrenal cortex of the normal dog than that of burned one.

Some of the pathways by which the

---

Formerly, Professor & Head, Department of Orthopaedic Surgery, R G Kar Medical College & Hospital; Residence : 16-B, Sachin Mitra Lane, Calcutta-700003.

adrenal is brought into increased activation after stress (fracture, burn and intestinal obstruction) were explored<sup>1</sup>. Both the nervous and vascular pathways are necessary for stress response. In a deafferented (both sympathetic and somatic) limb of a dog, the response can still be elicited after different types of stress. In midbrain sectioned dogs, the rise in 17-hydroxycorticosteroid output after stress is very small. Dogs with pituitary stalk section show increased adrenocortical activity (17-OHCS) after stress. Purely psychic stress requires the presence of the hypophysiportal vessels for the response. Atrophy of the neural lobe, ischaemic necrosis of the anterior lobe, enlargement of the pars intermedia and proximal stump of the stalk and median eminence were observed. Hypothalamic extracts from stressed dogs can increase adrenal venous 17-OHCS output in the assay animals (dogs) when injected into the carotid artery. The magnitude of response varies, depending on the type and duration of stress. A type of central control of ACTH discharge by histamine was suggested. Discharge of neurosecretory material (nsm) from the hypothalamic nuclei has been noted after stress. The substance has a role in adrenohypophysial ACTH discharge during stress. In pituitary stalk-sectioned animals if measures are adopted to prevent vascular regeneration, there is depletion of nsm from the proximal stump of the cut stalk after stress and the material enters into the systemic circulation and stimulates ACTH release. Diverse pathways by which the stress messages travel after burn to give rise to increased secretion of ACTH and adrenal hormones have been analysed. Some of the adrenocortical changes after burn in

humans, dogs and guineapigs include vacuolation and cytolysis, inversion of lipid pattern, lumina and tubule formation and congested appearance of the adrenals.

Roy<sup>2</sup> studied the maturation of hypothalamo-pituitary-adrenal axis. He found that nsm is not present in the destroyed or ill-formed neurohypophysis and hypothalamus of anencephalic foetus. The neural lobe may show multicystic appearance. Maternal stress is manifest in foetal adrenals.

Stress response in the form of activation of the pituitary-adrenal axis can be achieved after fracture and intramedullary pinning of long bones even in the absence of intact spinal cord in guineapigs<sup>3</sup>. The same stressors can lead to maximum adrenocortical activity in frontal leucotomized (FL) guineapigs. This is due to the fact that the hypothalamus is released of the inhibitory influences from the frontal cortex, leading thereby to increased ACTH secretion<sup>4</sup>.

Evaluation of resting adrenocortical secretion rates in non-stressed conscious dogs by collecting adrenal venous blood samples by the method of Hume and Nelson has been done by Roy<sup>5</sup>. Adrenal 17-OHCS secretion rate ( $\mu\text{g}/\text{min}$ ) was  $2.2 \pm 0.1$ . Ether anaesthesia in dogs without surgery leads to significant increase in adrenal venous 17-OHCS output at 30 and 60 minutes. Intravenous injection of pentobarbital sodium lowers the 17-OHCS output significantly in absence of surgery.

Experiments were conducted to understand brain mechanisms responsible for ACTH release in experimental burns<sup>6,7</sup>. The cerebral cortex of the dog has an inhibitory influence on the pituitary-adrenal



axis. After decortication operation, there is rise in adrenal venous 17-OHCS output. Further rise occurs after burn trauma. Brain removal up to the level of thalamus leads to increased adrenocortical response after burn. In solitary pituitary experiments with supratentorial brain matters removed, increased adrenocortical secretion has been noted with further rise after burn. The hind-brain-factor (HBF) is not very important for this increased response, which has been proved by removal of infratentorial structures as well.

Maximum adrenocortical response after burn appears late in dogs with anterior hypothalamic lesion and the magnitude of response is also less. Hippocampus, septum and the cingulate area have inhibitory influences over the pituitary-adrenocortical secretion. Lesion of these areas leads to increased response with further increase after burn trauma. Amygdala has a restraining influence and this may be due to the associated vascular lesion of the hippocampus. Adaptation activity also occurs at the brain level through different stimulatory and depressive areas of the brain and their afferent and efferent fibre systems. The net result is a steady state in the body in adverse situations, what Claude Bernard said long ago. Thus, apart from the endocrinal adaptation, the central neural integrations are also to be considered.

### Submammalian Vertebrates

#### Birds

The brain mechanisms responsible for ACTH release in the pigeon (*Columba livia*) were studied<sup>8</sup>. Corticotrophin releasing factor (CRF) is present in the median eminence. Increased ACTH release has been found after stimulation of

median eminence, nucleus hypothalamicus posteromedialis, nucleus inferior and arcuate nucleus (ventral and posterior hypothalamus) and archistriatum. Hippocampus, septum, septomesencephalic tract and medial forebrain bundle are inhibitory areas for ACTH release (lesion experiments). Stress responses have also been observed.

#### Lizards

Roy<sup>9</sup> studied the hypothalamo-pituitary-adrenal axis after stress in the garden lizard (*Calotes versicolor*). Stresses include fracture of both thigh bones, scald and ether anaesthesia. Nerve fibres containing nsm are found to end around the primary capillary net in the median eminence; nsm reaches the pars distalis through the hypophysis-portal vessels and a part contained in the material stimulates production of ACTH or gonadotrophin or other hormones. The hypothalamus, median eminence and the hypophysis show marked congestion after stress; nsm is also poured into the adjoining ventricle. Depletion of nsm occurred after stress. Inter-renal cells are small and contain lipids. Glandular hypertrophy occurs in summer, whereas during winter the reverse is true. Stress leads to congestion of the organ and loss of sudanophilic substance from the interrenal cells which lie ventrally in the organ and the adrenal element lies in the dorsal aspect of the gland. Scattered groups or solitary adrenal cells are found in the gland.

In *C. versicolor*, stimulation of median eminence, infundibular nucleus, ventromedial nucleus and archistriatum leads to increased blood corticosterone level; its fall was recorded after lesion of the same areas. Septal and hippocampal areas are inhibitory areas for ACTH

secretion. Active grafts of anterior pituitary have been noted when they are placed at the median eminence, infundibular nucleus and the recess of the third ventricle<sup>8</sup>.

### Amphibians

Roy<sup>10</sup> studied hypophysio-portal circulation and changes in hypothalamus, pituitary and adrenal of the toad (*Bufo melanostictus*) after fracture, formalin injection and scald. Vessels come out of the median eminence and form portal vessels which enter into the pars distalis and form secondary capillary net. The direction of flow is from the median eminence towards the pars distalis. The neurosecretory substance is present in the neurosecretory cell groups of the hypothalamus and passes along the preopticohypophysial tract to the neurohypophysis. After pituitary stalk-section, there is accumulation of nsm in the proximal stump (48 h); nsm may pass into the third ventricle. They have also been found to end in the blood vessels of the median eminence wherefrom they are carried to the pars distalis. Stress reduces nsm from the hypothalamic nuclei and neurohypophysis within 1 hour subsequent to congestion of the hypothalamus and pars nervosa. Replenishment of the substance occurs at varied intervals after 1 hour. Within the hypothalamic cells, there are appearances of vacuoles and the neurosecretory granules and colloids surround them. After stress, there is congestive appearance of the hypophysis and the adrenals. In the adrenals there is loss of sudanophilic substances. Schultz positive substance diminishes in the adrenal; the birefringent material also diminishes. Vacuolar change in cortical cells has also been noted. After stress,

cortical lipids are discharged and they help the animal to tide over the critical period.

A significant quantity of ACTH is not secreted by isolated toad hypophysis. In *Bufo melanostictus*, Roy<sup>11</sup> did not find corticosteroids in the blood of hypophysectomized toads or toads having ectopic autografts of the hypophysis. Measurable quantities were present in the blood of toads in which the hypophysis had been regrafted under the median eminence. Different types of stress, including constant illumination, bone fracture or scald, caused increase in blood corticosteroids to a considerable extent.

Stimulation and lesion experiments on the brain of toad (*Bufo melanostictus*) and lesion experiments on the brain of the garden lizard (*Calotes versicolor*) were conducted by Roy<sup>12</sup>. In *Bufo melanostictus*, electrical stimulation in the area of the preoptic nucleus, the ventral hypothalamus or the median eminence leads to raised plasma corticosteroids. The mean values obtained were 14.1, 12.8 and 15.5  $\mu\text{g}/100\text{ ml}$  plasma respectively. These values were significantly higher than in the shamstimulated control groups where plasma corticosteroids averaged about 6  $\mu\text{g}/100\text{ ml}$ . In the unoperated controls, it was 2.5  $\mu\text{g}/100\text{ ml}$ . Stimulation of the primordium hippocampi significantly reduced plasma corticosteroids to a level of 3.3  $\mu\text{g}/100\text{ ml}$ . Lesions in the preoptic area and specially in the ventral hypothalamus and median eminence reduced corticosteroid levels in the plasma. Lesions of the primordium hippocampi permanently increased corticosteroid levels (10.3  $\mu\text{g}/100\text{ ml}$  two weeks after the operation). In *Calotes versicolor*, the hippocampus has a checking influence over the pituitary-adrenal axis.



Similar observations were made by Roy<sup>8</sup> in the frog (*Rana tigerina*) by stimulation and lesion experiments. Inhibitory areas for ACTH release are hippocampus, septum and medial forebrain bundle. Stimulatory areas for ACTH release are amygdala, spinodiencephalic connections and infundibulum.

### Teleosts (*O. punctatus*)

ACTH, forced swimming, surgery and other forms of stresses were found to increase plasma 17-OHCS levels<sup>13</sup>. Significant increases in plasma 17-OHCS levels were observed after intraperitoneal injection of diencephalic and caudal neurosecretory extracts in *O. punctatus*. Changes after hypophysectomy were also noted. Gonadal atrophy was noted after lesions of hypothalamus and forebrain. Increased activity of the nucleus tuberis lateralis is noted during the breeding season. Mesoadenohypophysial gonadotrophs are stimulated by tuberal neurosecretion.

The effects of brain lesion and stimulation on ACTH secretion were studied in *Ophiocephalus punctatus*<sup>14</sup>. Lesions in different parts of the forebrain showed no permanent effects on plasma 17-OHCS levels. However, ablation of dorsal forebrain increased significantly plasma corticosteroid levels 1, 7 and 21 days after operation. The values reached normalcy one month after the operation. Electrical stimulation of the dorsal forebrain area reduced plasma corticosteroid level significantly. In the other regions of the brain, electrical stimulation either increased or did not change plasma corticosteroids. Forebrain ablation, including parts of the diencephalon, posterior to the optic chiasma, reduced corticosteroid levels

significantly even one month after the operation. The experiments suggest that neurons stimulating ACTH secretion have their origin in the anterior hypothalamus in *Ophiocephalus*. Changes in the pituitaries have been described by Roy<sup>15</sup>.

The caudal neurosecretory system of some Indian fishes was examined<sup>16</sup>. The teleosts examined had terminal swelling. In *Trygon sephen*, a type of chondropterygii-Trygonidae, there is no terminal swelling, but the junctional place between the neurosecretory axon and the blood vessel functions as the storage-release centre. In teleosts, the caudal neurosecretory system has got a cell station, axonal paths for migration of neurosecretory material (nsm) and the terminal swelling acting as the storage-release centre. The section of the caudal spinal cord in teleost leads to accumulation of nsm in the proximal stump, indicating proximodistal migration. There is also neoformation of the terminal swelling with atrophy of the original one. The proximal cells respond to stress. Corticotrophin releasing factor (CRF) has been isolated from the caudal neurosecretory system.

Stress response also occurs in invertebrates, though they do not possess the hypothalamo-pituitary-adrenal axis. This is a universal phenomenon<sup>8</sup>.

### Future Directions

The following are proposed to be the lines of future work:

(1) Different types of vertebrates and invertebrates are to be used for noting the stress response.

(2) In vertebrates, axes other than pituitary-adrenal axis are to be examined

using different stressors and different parameters.

(3) Use of electron microscope, immunocytochemistry, autoradiography, radioimmunoassay, and other methods will be made in stress research.

### Selected Publications

1. Roy B B, *Adrenocortical response in some surgical and experimental conditions*, 1959, pp 110.
2. Roy B B, Hypothalamo-pituitary-adrenal-axis and its maturation, *Indian med Forum*, **9** (1958) 227-37.
3. Roy B B, Effect of different types of fractures and operations (intramedullary pinnings) of bones on adrenals indicating the activity of the pituitary-adrenal-axis, *Indian med Forum*, **10** (1959) 171-84.
4. Roy B B, Frontal lobe of the brain and the adrenocortical response after fracture and operations on bones, *Indian med Forum*, **10** (1959) 218-25.
5. Roy B B, Effect of anaesthesia on adrenals, *Indian med Forum*, **20** (1969) 91-98.
6. Roy B B, Brain mechanisms responsible for ACTH release in experimental burns—Part I, *Proc natn Inst Sci India*, **32B**(5 & 6) (1966) 243-71.
7. Roy B B, Brain mechanisms responsible for ACTH release in experimental burns—Part II, *Calc med J*, **64** (1967) 298-339.
8. Roy B B, *Neuroendocrinological studies in stress (Experimental surgical observations in vertebrates and invertebrates)* (University of Calcutta) 1976, pp 518.
9. Roy B B, The hypophysis-portal circulation, hypothalamus, pituitary and adrenal in *Calotes versicolor* and changes in hypothalamus-pituitary-adrenal-axis in fracture and other forms of stresses, *Indian med Forum*, **9** (1958) 51-54.
10. Roy B B, Hypophysis-portal circulation, hypothalamus, pituitary and adrenal of the *Bufo melanostictus* and changes in the hypothalamus, pituitary and adrenal after fracture and other stresses, *Indian med Forum*, **8** (1957) 1-4.
11. Roy B B, Environment, stress and adrenocortical response, *Antiseptic*, **57** (1960) 719-39.
12. Roy B B, Brain mechanisms responsible for ACTH release in the toad—*Bufo melanostictus* with a note on *Calotes versicolor*, *Bull Univ Coll Med, Calcutta*, **7,8** (1969-71) 1-13.
13. Roy B B, Production of corticosteroids *in vitro* in some Indian fishes with experimental, histological and biochemical studies of adrenal cortex together with general observations on gonads after hypophysectomy in *O. punctatus*, *Calc med J*, **61** (1964) 223-44.
14. Roy B B, Brain mechanisms responsible for ACTH release in the fish, *J Inland Fish Soc India*, **1** (1969) 13-31.
15. Roy B B, *Comparative aspects of the pituitary gland* (University of Calcutta) 1981, pp 652.
16. Roy B B, Histological and experimental observation on the caudal neurosecretory system of some Indian fishes, *Proc natn Inst Sci India*, **28B**(5) (1962) 449-77.



## A K Saha

The second of the series of papers published by Saha in 1950 in the *Indian Journal of Surgery* was reprinted in *Clinical Orthopaedics and Related Research* (No. 173, March 1983) as a 'classic', describing it as "fundamental research".

In 1950, a technique of twin L shaped or single biflanged angulated graft from the tibia for intracapsular fracture of neck of the femur using specially designed instruments for their introduction was developed. This was done at a time when the failure rate with Smith Petersen nail was 30%.

Several papers dealing with shoulder research on the management of flail hip and conservatism in limb surgery in malignant and borderline malignant tumours in long bones were published in various journals, including the *Journal of the International College of Surgeons*. All the articles on surgical rehabilitation of flail hip were summarized in the yearbooks of Orthopaedics and Plastic Surgery published in USA.

In collaboration with the National Institute and Government Test House (Government of India), Zoology, Anthropology and Physiology Departments of Calcutta University, Radiology Department of Chittranjan Seva Sadan

and NRS Medical College and Hospitals research work on comparative morphology and anatomy and on muscle function of pectoral girdle and glenohumeral joint, using among other procedures, 8 channel electromyography (on a modified electroencephalograph) were done in the early fifties.

In 1957, I was awarded the Hunterian Professorship of the Royal College of Surgeons, UK, and at the same time was invited to lecture in several other universities in UK and USA, Veterans Hospital and Air Base Hospital in Omaha.

In 1961, the first monograph on '*Theory of shoulder mechanism—Descriptive and applied*' was published with a foreword by Prof. Edward L Compere. Prof. Sten Friberg of Karolinska Institute, Sweden, in a personal letter to me commended it as a forerunner of many new ideas on the shoulder joint.

In 1967, *Acta Orthopaedica Scandinavica* published my second monograph as a supplement and an article on recurrent anterior dislocation of the shoulder. The concept of dynamic stability of the shoulder in the rehabilitation of the paralysed and flail shoulder and recurrent anterior dislocation was thus established. This forms the most important guideline in formulating the surgical procedures.

In 1969, the monograph '*Recurrent anterior dislocation of the shoulder: A new concept*' was published. This monograph deals with morphological, anatomical and functional aspects of the shoulder joint.

As many as over 100 operations were practised empirically for recurrent anterior shoulder dislocation with functional recovery and recurrence beyond expectation. In a joint with normal glenohumeral index, these were reduced to three new operations—Latissimus dorsi transfer, derotation osteotomy of upper humerus and glenoid osteotomy, which were devised to restore the dynamic stability of the joint. Depending on the indications through proper screening, the operations restore dynamic stability and give full function with no recurrence.

Though I had been a member of SICOT since 1952, for the first time I attended and took active part in the triennial congress at Mexico in 1969 at the invitation of Prof. Sten Freiberg, Rector of Karolinska Institute and the then President of SICOT.

In 1975, I presented my work on physiological total shoulder replacement at the triennial congress in Copenhagen starting with all-metal prostheses without cement. The function of the shoulder was restored by the transplanted steerers to a metal replica of the head. This was incorporated as a problem in the German edition of my monograph published by Ferdinand Enke, Verlag. The method has been changed by Dr S K Dutta to a metal-plastic prosthesis using cement.

In 1983, I was invited as the first guest lecturer at the second annual conference of the American Shoulder and Elbow Surgeons affiliated to the American

Academy of Orthopaedic Surgeons, to deliver a lecture on "Theory of shoulder mechanism—Descriptive and applied". They made me a corresponding member in recognition of "outstanding contribution in the field of shoulder surgery".

Currently, we are carrying out work on theoretical aspects of the shoulder girdle and the dynamical basis for Latissimus dorsi transfer in the treatment of recurrent dislocation of the shoulder.

### Selected Publications

1. Saha A K, Mechanism of shoulder movements and a plea for the recognition of zero-position of the glenohumeral joint, *Indian J Surg*, **12** (1950) 153.
2. Saha A K, Das N N & Chakraborty B G, Studies on electromyographic changes of muscles acting on the shoulder joint complex, *Calc med J*, **53** (1956) 409.
3. Saha A K, Saha M R & Chakraborty B G, Anatomical and mechanical observation on glenohumeral joint, *Calc med J*, **54** (1957) 48.
4. Saha A K, Zero-position of the glenohumeral joint: Its recognition and clinical importance, *Ann R Coll Surg UK*, **22** (1958) 223.
5. Das S P, Ray G S & Saha A K, Observations on the tilt of the glenoid cavity of scapula, *J anat Soc India*, **15** (1966) 114.
6. Saha A K & Das A K, Anterior recurrent dislocation of the shoulder: Treatment by rotation osteotomy of the upper shaft of the humerus, *Indian J Orthop*, **1** (1967) 132.
7. Saha A K, Dynamic stability of glenohumeral joint, *Acta orthop scand*, **42** (1971) 491.
8. Saha A K, Mechanism of elevation of glenohumeral joint etc., *Acta orthop scand*, **44** (1973) 668.
9. Chaudhuri G K, Sengupta A & Saha A K, Rotation osteotomy of the shaft of the humerus for recurrent dislocation of the shoulder: Anterior and posterior, *Acta orthop scand*, **45** (1974) 193.
10. Saha A K, Bhattacharjee D & Dutta S K, Total shoulder replacement: A preliminary report, *J Indian med Ass*, **66** (1976) 121.
11. Saha A K, Biomechanics of the glenohumeral joint: Mathematical treatment, *Proc Indian natn Sci Acad*, **44A** (1978) 195.



12. Saha A K & Bhadra N, Mechanics of the movements of the shoulder girdle, *Proc Indian natn Sci Acad*, **49A** (1983) 586.
13. Saha A K, *Theory of shoulder mechanism: Descriptive and applied* (Thomas, Springfield, Illinois) 1961.
14. Saha A K, Surgery of paralysed and flail shoulder, *Acta orthop scand* (Suppl), **97** (1967).
15. Saha A K, *Recurrent anterior dislocation of the shoulder: A new concept* (Academic Publishers, Calcutta) 1969.
16. Saha A K, *Rezidivierende schulterluxation: Pathophysiologie und operative korrektur* (Ferdinand Enke, Verlag, Stuttgart) 1978.
17. Saha A K, *Recurrent dislocation of the shoulder: Physiopathology and operative corrections* (Georg Thieme, Verlag, Stuttgart; Thieme-Stratton Inc, New York), 1981.

## J K Sarkar

### (I) Viral Diseases

#### (A) Smallpox

(1) Previously it was thought that the vaccination status of the individual was the only factor determining the severity of the case. It was shown for the first time that the virulence of smallpox virus (variola) differs in different strains and an experimental model to test the difference in virulence was developed<sup>1</sup>.

(2) The minimum protective level of antibody in smallpox was assessed for the first time<sup>2</sup>.

(3) For the first time it was demonstrated clinically and virologically that smallpox and chickenpox can occur simultaneously in the same patient<sup>3</sup>.

(4) It was established for the first time that asymptomatic household contacts of smallpox patients can spread smallpox<sup>4</sup>.

(5) Live virus of sufficient titre was shown to be excreted in urine and conjunctival secretion of smallpox cases<sup>5</sup>.

(6) For selecting the type of vaccine for use in India, a comparative study of Russian freeze-dried vaccine and Indian cow-calf lymph was made<sup>6</sup>.

(7) Mosquitoes were shown to have limited role in the spread of smallpox<sup>7</sup>.

(8) The pattern of intrafamilial transmission of smallpox in Calcutta was worked out<sup>8</sup>.

(9) The missing link between seasonal and off-season smallpox in Calcutta was established<sup>9</sup>.

The other major topics covered include: Viraemia and antibody response in haemorrhagic and other forms of smallpox; Relationship between neutralizing and haemagglutination-inhibiting antibodies in smallpox; Clinical, virological and serological studies on monkey after intravenous inoculation of virulent variola virus; Virus content of smallpox scabs; Evaluation of the precipitation-in-gel reaction in the diagnosis of smallpox; Serum proteins and antibody studies in smallpox; Relationship of clinical severity, antibody level and previous vaccination status in smallpox; Influence of certain bacteria on the growth of variola and vaccinia viruses; and Difference between the behaviours of variola and vaccinia viruses in human amnion cell culture. The first case of virologically proved generalized vaccinia was reported from India. Some suggestions were made to WHO on the post-eradication state of smallpox<sup>10</sup>.

---

Formerly, Professor of Virology and Director, School of Tropical Medicine, Calcutta; Residence : 87, Golf Club Road, Tollygunge, Calcutta-700033.



**(B) Dengue Haemorrhagic Fever (DHF)**

(1) A mouse model to study DHF was established<sup>11</sup>. Some workers had tried this in other animals but had not achieved success.

(2) The first DHF outbreak in Calcutta (also in India) in 1963 was investigated and the causative virus isolated<sup>12</sup>.

(3) Chikungunya virus was isolated in India for the first time and it was demonstrated again for the first time that this agent can cause haemorrhagic fever<sup>13</sup>. This fact was later confirmed by other workers.

(4) The first case of dengue encephalitis in India, proved virologically, was reported<sup>14</sup>.

Other studies conducted include: Susceptibility of new born and adult laboratory animals to Chikungunya virus; Re-survey for arbovirus antibodies in human sera after an interval of six years; Sporadic cases of DHF during dengue epidemics; Transplacental transmission of haemagglutination-inhibiting antibodies of pox and arboviruses; Experimental studies on dengue type-2 infection of mice after infection of dengue type 1 antiserum; and Observation on second heterotypic dengue virus infection in mice. The last two studies served to explain the pathogenesis of dengue haemorrhagic fever.

**(C) Japanese Encephalitis**

(1) The first epidemic of Japanese encephalitis (JE) in India was established virologically<sup>15</sup>. This incidentally was the first encephalitis epidemic in India to be diagnosed virologically. Not only were the vector mosquitoes in West Bengal region identified, but isolation of the virus from

two species of anopheles mosquitoes was a revelation.

(2) The efficacy of Japan-made JE vaccine in providing protection against infection by the local strain was proved<sup>16</sup>.

The factors leading to recurrent JE in West Bengal were investigated.

**(D) Other Virus Diseases**

(1) Strains of rubella virus were isolated from cases for the first time in India<sup>17</sup>. Also, rubella antibody profile in women of child bearing age and rubella as an aetiological factor of congenital malformation in Calcutta were studied.

(2) Coxsackie A4 was found to be one of the aetiological factors of encephalitis in children of Calcutta. Also, coxsackie virus infection simulating smallpox was reported.

**(II) Bacterial Diseases**

(1) *Alkaligenes faecalis* was shown to be a common pathogen in Calcutta; systematic study of this organism was made in 1959.

(2) The growth and survival of *V. cholerae* in water and in relation to pH was studied. It was shown that a loopful of *V. cholerae* can eliminate all *E. coli* in broth culture in 5-6 days. Antagonism between *E. coli* and common intestinal pathogens was shown.

(3) In Calcutta, the pathogenicity of *C. diphtheriae* mitis was shown to be very high.

(4) A new type of propionibacterium was isolated from lepromatous nodules and from deep layers of skin of some normal persons. It was suggested that the

organism may have aetiological bearing on the disease<sup>18</sup>.

(5) The first paper in India on pathogenic *E. coli* was published<sup>19</sup>.

### (III) Other Studies

(1) A tissue culture model for testing anti-microfilarial drugs was demonstrated<sup>20</sup>.

(2) The first paper in India on antibiotic resistant bacteria was published<sup>21</sup>. Also, penicillin inactivation by sera of patients previously treated with penicillin was reported in 1961.

(3) The effects of variola, vaccinia and coxsackie viruses on the mitosis of cultured human cells were studied in detail. The effect of Sarcoma 80 on the mitosis of duodenal epithelium of mice was reported.

(4) Methodology for determining the potency of anti-snakevenom serum was standardized.

(5) Three separate outbreaks of tricresyl phosphate poisoning in different years were studied and diagnosed.

(6) Immunization against Sarcoma 80 was shown to be possible in mice<sup>22</sup>.

### Selected Publications

1. Sarkar J K & Mitra A C, Virulence of variola virus from smallpox cases of varying severity, *Indian J med Res*, **55** (1967) 13.
2. Sarkar J K, Mitra A C & Mukherjee M K, The minimum protective level of antibodies in smallpox, *WHO Bull*, **52** (1975) 307.
3. Sarkar J K, Mitra A C, Mukherjee M K, Dumbell K R & Almeida J D, Concurrent smallpox and chickenpox, *WHO Bull*, **54** (1976) 119.
4. Sarkar J K, Mitra A C, Mukherjee M K, De S K & Guha Majumdar D, Virus excretion in the throat of household contacts of smallpox patients, *WHO Bull*, **48** (1973) 523.
5. Sarkar J K, Mitra A C, Mukherjee M K, De S K & Guha Majumdar D, Virus excretion in the throat, urine and conjunctiva of patients, *WHO Bull*, **48** (1973) 517.
6. Sarkar J K, Chatterjee S N & Chakravarty S K, Comparative study of Russian freeze-dried smallpox vaccine and an Indian cow-calf lymph, *Indian J med Res*, **52** (1964) 241.
7. Sarkar J K, Hati A K & Mitra A C, Role of mosquitoes in the spread of smallpox, *J infect Dis*, **128** (1973) 781.
8. Mukherjee M K, Sarkar J K & Mitra A C, Pattern of intrafamilial transmission of smallpox in Calcutta, *WHO Bull*, **51** (1974) 219.
9. Sarkar J K, Ray S & Manji P, Epidemiological and virological studies on the off-season smallpox cases in Calcutta, *Indian J med Res*, **58** (1970) 829.
10. Sarkar J K, Telling the truth about smallpox eradication, *Wld Hlth Forum*, **5** (1984) 241.
11. Sarkar J K, Das B C, Mukherjee K K & Chakravarty S K, Observation on second heterotypic dengue virus infection in mice, *Indian J med Res*, **64** (1976) 1713.
12. Sarkar J K, Chatterjee S N & Chakravarty S K, Three year study of haemorrhagic fever in Calcutta, *Trans Soc trop Med Hyg*, **61** (1967) 725.
13. Sarkar J K, Chatterjee S N, Chakravarty S K & Mitra A C, Chikungunya virus infection with haemorrhagic manifestations, *Indian J med Res*, **53** (1965) 921.
14. Sarkar J K, Mondal A, Chakravarty S K, Chatterjee S N & Pal S R, Isolation of dengue virus from the blood of clinical case of encephalitis, *Indian J med Res*, **57** (1969) 1616.
15. Chakravarty S K, Sarkar J K, Chakravarty M S, Mukherjee M K, Mukherjee K K, Das B C & Hati A K, The first epidemic of Japanese encephalitis studied in India, *Indian J med Res*, **63** (1975) 77.
16. Das B C, Chakravarty S K, Sarkar J K & Mukherjee K K, Comparative study of protection by Japanese encephalitis (JE) vaccine against a Japanese strain and an Indian strain of JE virus, *Indian J Path Microbiol*, **19** (1976) 239.
17. Sarkar J K, Mukherjee M K, Chakravarty M S, Roy I, Bose A K, Chowdhuri A, Das B C, Das S K & Chakravarty S K, Virologically proved rubella cases in Calcutta, *J Indian med Ass*, **63** (1974) 194.
18. Sarkar J K, Ghosh S & Chatterjee B D, New types of propionibacterium from lepromatous



- modules and deep layers of normal skin, *Indian J med Res*, **71** (1980) 354.
19. Tribedi B P & Sarkar J K, Serological study of *Bacterium coli* in diarrhoea in relation to its probable role as pathogen, *Indian J med Sci*, **9** (1955) 516.
20. Sarkar J K, A tissue culture model for testing anti-microfilarial drugs, *Trans R Soc trop Med Hyg*, **63** (1969) 374.
21. Tribedi B P & Sarkar J K, Bacterial resistance to antibiotics, *J Indian med Ass*, **23** (1954) 324.
22. Sarkar J K & Chakravarty S K, Immunisation against Sarcoma 180 in Swiss mice, *Indian J Cancer* (1968) 206.

## S C Seal

Seal has been engaged in research work in medical and health sciences for the last 55 years since his graduation in 1929. He has carried out this work in his successive assignments as clinical house staff (1930-1932), Medical Research Worker, Biological Division, B C P W. Ltd, Calcutta (1932-34), Senior Bacteriologist, Cholera Research, IRFA (1934-1937), Field Epidemiologist, Cholera Carrier Inquiry (1937-39), Rockefeller Foundation Fellow (1939-40), Medical Research Officer, Plague Researches Inquiry, Haffkine Institute, Bombay (1941-43), Assistant Professor and later Professor of Epidemiology, All India Institute of Hygiene and Public Health, Calcutta (1943-1960), Officer on Special Duty, Morbidity Survey, DGHS, New Delhi (1960-1964), Professor and Head of the Department of Medical Social Work and later Professor of Public Health Administration, Urban Administration Course, Indian Institute of Social Welfare and Business Management, Calcutta (1964-1969), Emeritus Medical Scientist, ICMR (1970-1973), Director, India Fertility Research Programme, Calcutta (1973-75), and Consultant, Health Cell, Calcutta Metropolitan Development Authority (1976-81). He could thus cover a wide range of subjects of study.

As a house staff he studied splenomegalic cirrhosis of liver, snake-bite neuritis, aneurysm of aorta, and

bronchogenic carcinoma as the first authentic case reported in medical literature in India. In the BCPW Ltd, he worked on bacterial derivatives for active immunization, antigenic constitution of *E. typhosus*, and studied an epidemic of meningococcus meningitis. During this period, he undertook the study of history and epidemiology of epidemic dropsy and continued to maintain his interest in it till 1952. In cholera research, he was associated with Dr R W Linton for working out the chemical antigenic structure of cholera vibrios by single cell culture. With Dr Lal he studied human carrier of cholera and with Major Read, the environmental sources of vibrios in connection with the endemicity of cholera in the Sunderban area of Khulna district (of old Bengal).

His most outstanding work is on plague, done at the Haffkine Institute, Bombay and at Calcutta in the Department of Epidemiology, during Calcutta plague between 1948 and 1952 and also afterwards. He was able to study all aspects of the problem. During this period, apart from his contribution to plague and teaching in epidemiology in India with many new innovations, he took the opportunity to study all kinds of epidemics in India. He also contributed to medical education, study of history of medicine and public health in India and of certain specific diseases, social and preventive



medicine, fertility research and population control and in planning and evaluation work.

He has to his credit pioneering work in developing a method for carrying out an integrated health, socio-economic and morbidity survey in India. He has himself carried out as many as 25 such surveys; the most important and outstanding ones are that of CHS beneficiaries in Delhi and New Delhi areas (4.5 lakh population) and the other among 9.6 million population in the CMDA area, including surveys on mental health and medical care services in the area.

The total number of his contributions is now 500 (including some yet to be published). A brief review of Seal's important researches and publications is given below.

### **Diphtheria and Gas Gangrene Toxins**

While in USA and Canada as a Rockefeller Foundation Fellow, he solved two local problems. The first problem in USA was the adverse reaction following the second dose of alum-precipitated diphtheria toxoid. This was overcome by preparing a high Lf toxin in a newly discovered protein-free medium and eluting the toxoid in a phosphate buffer. The second problem which was posed by the Connaught Laboratories of Toronto was the absence of an assessment method for the antigenic value of gas gangrene toxoid. Seal discovered a new medium for anaerobic culture of the organism *C. perfringens* yielding a highly potent gas gangrene toxin and utilized the human serum opacity test for estimating the antibody binding power of the toxoid by quantitative complement-fixation test.

### **Plague**

Despite some knowledge about causative germ, clinical condition and epidemiological features of plague, it was incomplete in respect of the cultural and serological characteristics of the organism as well as its antigenic structure and treatment, till 1940. As the organism was auto-agglutinable, confirmatory diagnosis of plague depended mainly on the time-consuming animal inoculation test; the same was true of the assessment of immunity following vaccination or antiserum production in horses. These difficulties were overcome by Seal by discovering a proper culture medium—casein hydrolysate broth, which facilitated isolation of the specific soluble protein as its chemical antigenic substance in pure form. He then studied various other aspects of the problem, namely, morphology, variation, identification, classification, serology, toxicity and virulence of the organism, differentiation with allied organisms, transmission, role of rodents and fleas, epidemiology, interepidemic phase, treatment, production of the most effective vaccine, and antiserum, surveillance, prevention and control. He contributed 50 papers and a monograph entitled "*Eradication of Plague in India*".

### **Cholera**

As a senior bacteriologist under Dr Linton between 1934 and 1937, Seal showed better agglutination reaction with live suspension and the value of '0' agglutination in identification of the non-cholera vibrios from the cholera group in 1935 simultaneously with that of Gardner and Venkataraman whose serological classification was officially accepted for identifying cholera vibrios.

This resulted in the rejection of Linton's chemical antigenic classification, as it showed relationship of Eltor with cholera vibrios as also of certain vibrios of NAG group, although Seal showed dissociation of the vibrio and reversion back to the original form by single cell culture. While working as field epidemiologist under Dr Lal (1937-39), he also found Eltor and certain NAG vibrios as carrier organism in human subjects. Now it is well known that Eltor is cholera vibrio and also acts as a carrier organism in both epidemic and endemic situations and is responsible for epidemics and pandemics. Seal has also worked on all other aspects of the cholera problem in India, namely, study of endemic and epidemic situations, treatment, prevention and control. His latest work is on the production of true cholera *exotoxin* by anaerobic culture of the organism, facilitating preparation of the most effective toxoid vaccine as the most potent measure towards control and finally eradication by tackling man and not depending on the sanitation of environmental sources which get repeatedly contaminated by human carriers and active cases. Seal contributed 41 papers in this field.

### **Epidemic Dropsy**

Even after the pathology of this disease, prevalent in the eastern region of India, had been worked out, controversy persisted in respect of its etiology. Seal's extensive work on its history and epidemiology in 1936 provided the clue to suspect mustard oil. Human experimental study by Dr Lal finally led to the finding that *Argemone mexicana* oil provides the toxic component when it is used as an adulterant for mustard oil. Although Dr

Sarker's report that the alkaloid sanguinarine is the said toxic component could not be confirmed by Seal, he showed that the toxic oil could be detoxicated by dry phosphoric acid or by exposure to sunlight.

### **Smallpox**

Prior to the eradication of this disease, Seal was associated with several assessment studies pertaining to this disease and with the formulation of plans for its eradication. His special contribution is on its immunological aspect. He found that cases were largely occurring among the vaccinated people than among the unvaccinated ones which constituted hardly 5-10% of the population. Using the serological method devised by him, he showed that when the immunity level of those once vaccinated came down below the dilution of 1:20, they became victims to infection when exposed and that all persons vaccinated did not respond to a high level of immunity after primary vaccination. Following an assessment of the Delhi Eradication Programme he not only detected pockets left out of revaccination but swelling of figures by repeat revaccination of the same persons more than once. He suggested that the coverage should be 100% instead of 80% as suggested by the planners. Ultimately success was attained when WHO accepted and implemented 100% coverage.

### **Tuberculosis and Leprosy**

Seal studied the urban-rural relationship in the spread of tuberculosis and leprosy and found them to be as much indigenous to urban areas as to the rural areas. He has been assessing the effect of treatment of leprosy during the last 50 years on the incidence and type of leprosy in West



Bengal. He is also interested in the current multi-drug trials.

### Virological Infections

Seal was the first person to identify the cause of Jamshedpur epidemic of 1954 to be Japanese B encephalitis virus, but it could not be isolated at that time. This epidemic was also noted in several other towns in Bihar, UP, Delhi and Madhya Pradesh. His investigation in Nagpur also led to the same conclusion. In both the instances, antimosquito measures brought the disease under control. The virus was, however, isolated by the Virus Laboratory, Poona, a short while after these incidences, from the mosquito, *C. vishnui*, in South India and from 1973 onwards in cases and mosquitoes by the School of Tropical Medicine, Calcutta.

Among other epidemiological studies on viral diseases conducted by Seal, mention may be made of poliomyelitis in the Andaman Islands, infective hepatitis in Gomoh Railway Colony and dengue haemorrhagic fever.

### Malaria

On the clinical aspect, Seal reported cases of peripheral nerve palsy due to *P. falciparum* infection and allergy due to *P. vivax*, cured by anti-malaria treatment. Another interesting observation was in regard to the breeding habits of anopheline mosquitoes. These avoided the village tanks inundated by flood water (silting) and those receiving bamboo leaves from the groves lying on the sides of the tank. He also discovered the healthy gametocyte carrier prior to the rise of malaria epidemic following mosquito breeding after the rains. In respect of the eradication programme, he warned the planners that exclusion of hypoendemic areas and cities

and towns from the purview of DDT spraying will initiate return of malaria, which has come true. He is currently studying such an epidemic in Calcutta.

### Fertility Research

As Director of India Fertility Research Programme, Seal involved a large group of senior gynecologists all over India in this research programme. He also established the Calcutta Family Welfare Hospital for fertility research and himself participated in fertility research in that hospital, the most outstanding work being improvement of MR technique and successful female sterilization by the non-surgical quinacrine instillation method. Out of 1000 cases done so far the follow up of 400 cases has been completed for two years. The failure rate noted is only 4%, with no tangible side-effects or complications. In collaboration with Dr Datta, he studied cases of sterility among rural women. Incompatible blood groups among the married couple was found to be a significant cause.

### Health and Morbidity Surveys

Designing of an integrated health, socio-economic and morbidity survey was a pioneering contribution of Seal; he standardized it to assess the health condition of a community or to evaluate the progress of any health scheme introduced into an area. The method has been used extensively for a base-line survey before a plan is introduced (such as in Community Development Projects); the results are reassessed through a resurvey a few years later. This exercise has been utilized extensively in the training of health workers and postgraduate students at the All India Institute of Hygiene and Public Health as well as of the Indian Institute of

Social Welfare and Business Management, Calcutta. The first survey was done in the Singur Health Centre area in 1943-44 followed by that of Sikkim state and community development projects in nine states in India, and resurvey of the Singur Health Centre Area. Altogether 25 such surveys have been conducted under the supervision of Seal, the two most important ones being that of CHS beneficiaries (4.5 lakh) in Delhi and New Delhi areas and of 9.6 million population of the Calcutta Metropolitan Development Authority. The second survey also included mental health and medical care facilities in the areas as well as a comparative study between improved and unimproved bustees in Calcutta. Seal also suggested an annual sample survey on all India basis to provide reliable vital statistical data for planning purposes. It has been incorporated in Mudaliar Committee's Report (1963).

### Planning

Seal actively participated in planning for establishment of institutions, health services, urban and rural health centres, relief and rehabilitation of refugees, medical social service in hospitals, dental care services in India, Central Institute of Epidemiology and Vital Statistics, National Institute of Communicable Diseases, Teaching Curriculum for Preventive and Social Medicine, Epidemiology and National Control of Smallpox and Cholera and control of communicable diseases like tuberculosis, leprosy, filaria, etc.

### Occupational Health

Seal conducted several studies on Indian seamen's health and hazards in collaboration with the medical officers of Port Health, Calcutta. He is currently

conducting an assessment of health conditions of colliery workers in Asansol area.

### Mental Health

Seal conducted a preliminary survey of mental health conditions of certain localities in Calcutta to plan for a comprehensive survey of mental health conditions in the area covered by the Calcutta Metropolitan Development Authority. This has been subsequently done in collaboration with Prof. Ajita Chakrabarti.

### Selected Publications

1. Chakravarty A K & Seal S C, Factors influencing natural immunization in diphtheria in urban and rural communities in West Bengal, *Indian J med Res*, **62** (1974) 178.
2. Seal S C, Studies on plague and allied organisms, *Thesis, University of Bombay*, 1947.
3. Seal S C, Isolation of active polysaccharide fraction from Haffkine plague vaccine, *Proc Soc Biol exp Med*, **77** (1951) 675.
4. Wagle P M & Seal S C, Application of DDT, BHC and cyanogas in the control of plague in India, *Bull Wld Hlth Org*, **9** (1953) 597.
5. Seal S C, Studies in immunization against plague—Application of anti-plague serum, *Ann Biochem exp Med*, **14** (1954) 9.
6. Seal S C, A comparative study of historical evolution, microbiology, immunology, epidemiology, surveillance, treatment, prevention and control of plague, *DSc thesis, University of Bombay*.
7. Linton R W & Seal S C, The effect of using living or dead suspension of vibrio on the agglutination titre, *Indian med Gaz*, **76** (1935) 68.
8. Linton R W, Shrivastava D, Mitra B N & Seal S C, Studies on the antigenic structure of *V. cholerae*; Parts VIII and IX, *Indian J med Res*, **22** (1935) 617; 635.
9. Shrivastava B L & Seal S C, Preparation and properties of specific polysaccharide from a strain of *V. cholerae*, *Proc Soc exp Biol Med*, **36** (1937) 157.
10. Seal S C, Sulphaguanidine in the treatment of cholera under field conditions, *J Indian med Ass*, **17** (1943) 85.



11. Seal S C, Ghosal S C & Ghosh M N, Trial of Aureomycin (IV) in the treatment of cholera, *Indian med Gaz*, **86** (1951) 287.
12. Seal S C, The known and unknown about cholera vibrio, *Calc med Rev*, **18** (1957) 63.
13. Seal S C, Primary bronchogenic carcinoma, *Indian med Gaz*, **67** (1934) 237.
14. Seal S C, Epidemic dropsy and mustard oil, *J Indian med Ass*, **10** (1941) 91.
15. Seal S C & Roy D K, Immunization studies in smallpox, *Indian J med Res*, **56** (1968) 467.
16. Seal S C, Influence of environmental factors in the incidence of tuberculosis, *J Bengal Tuberc Ass*, **18** (1955) 56.
17. Seal S C, Epidemiological approach to the control of tuberculosis, *J Tuberc Ass India*, **27** (1972) 63.
18. Seal S C & Sen U, The problem of urban leprosy, *Proc natn Conf on Communicable Diseases*, Jhansi, February 1976.
19. Seal S C, Leprosy—Its prevention and control, *Calc med J*, **81** (1984) 113.
20. Seal S C, A note on the outbreak of encephalitis (J.E.) in Nagpur City, *Indian J publ Hlth*, **3** (1959) 147.
21. Seal S C, Changing pattern of diseases and the problem of their control, *Indian J publ Hlth*, **2** (1958) 143.
22. Seal S C, Bengali diet with special reference to metabolism and significance in maintenance of health, *Calc med J*, **36** (1939) 390.
23. Saha A L & Seal S C, Diet and nutritional pattern of certain rural areas in India, **3** (1965) 8.
24. Seal S C, Organization of medical education to meet the changing needs of the society, **47** (1966) 459.
25. Seal S C, Current thoughts on medical and public health education, *Indian J publ Hlth*, **22** (1978) 289.
26. Bhattacharji S C, Banerji G L & Seal S C, Enterogastric syndrome as an occupational hazard in the Merchant Navy at the Calcutta Port, *Indian J publ Hlth*, **15** (1971) 43.
27. Seal S C, Bose F N & Ghosal S C, Notes on bamboo flowering, migration of rats and their unusual mortality in certain districts of North Assam, *Calc med J*, **48** (1951) 199.
28. Seal S C & Stewart S E, Application of human serum opacity reaction for evaluation of the anti-toxin binding power (Lb) of *Cl. perfringens* (type A) toxoid, *Publ Hlth Rep (Washington)*, **56** (1941) 792.

## A B Sen

Sen is one of India's leading medical parasitologists. He has made substantial contributions to chemotherapy and immunology<sup>1,2</sup> of several tropical diseases of national priority and has been the pioneer in using *Mastomys natalensis* as a novel host for various helminthic and protozoal infections.

More specifically, he has discovered a new filarial species *Chandlerella hawkingi* from Indian Jungle Crows with sheathed microfilariae and nocturnal periodicity and elucidated the mechanism of its periodicity<sup>3,4</sup>. He demonstrated the relationship of adverse reactions to diethylcarbamazine in microfilaraemic subjects with sudden release of massive amounts of antigen in the already sensitised host<sup>5,6</sup>.

Sen and his colleagues have developed a specific and sensitive diagnostic skin test for lymphatic filaria using infective larval antigen of *B. malayi*<sup>7,8</sup>. Extensive clinical studies have established its validity and suitability. Their introduction of hamster adapted *Ancylostoma ceylonicum* as a 'screen' has proved to be very useful.

Sen has developed a battery of test systems for better anti-protozoal and anti-helminthic drug screening and has screened many synthetic compounds and natural products<sup>9-12</sup>. He holds 23 patents on these compounds. A new antifilarial

compound, Centperazine, whose activity was discovered by his group, is undergoing extensive clinical studies, while two other broad spectrum anthelmintic compounds are under preclinical safety evaluation.

Sen has made important contributions to immunology and related basic aspects of several protozoal disorders, particularly malaria<sup>13</sup>. He has perfected a screening technique for casual prophylactic agents employing *P. berghei* infection in mastomys. Even more exceptional has been his successfully culturing erythrocytic stages of *P. berghei* *in vitro* in mastomys erythrocytes<sup>14</sup>. His investigations for the first time demonstrated kinins in plasma of guineapig infected with *Trypanosoma evansi*<sup>15</sup>. More recently, he has standardized a technique for laboratory studies on leishmania and established *Leishmania donovani* infection in *Mastomys natalensis* in addition to hamsters and BALB/C mice *in vivo*. With his colleagues, he has developed and standardized a technique for *in vivo* screening of potential antileishmanials. He has used cotton rat peritoneal macrophages for *in vitro* culture of amastigotes, the human form of this parasite for studying the mode of action of antileishmanials.

Sen has published more than 130 original research papers in various national

---

Deputy Director & Head, Division of Parasitology, Central Drug Research Institute, Lucknow-226001; Residence : F1, CSIR Colony, Nirala Nagar, Lucknow-226007.



and international scientific journals of repute and has jointly edited a book entitled "*Chemotherapy and immunology in the control of malaria, filariasis and leishmaniasis*" with Dr Nitya Anand (Tata McGraw-Hill Publishing Co. Ltd, New Delhi, 1983). He is presently editing an annual series "Perspectives in Parasitology" jointly with Drs J C Katiyar and P Y Guru (Print House, Lucknow). Its first volume has already been published.

### Selected Publications

1. Katiyar J C, Gupta S & Sen A B, Action of diethylcarbamazine citrate on protective immunity in rats infected with *Nippostrongylus brasiliensis*, *Z Parasitenk*, **71** (1985) 401-8.
2. Tyagi K, Murthy P K & Sen A B, Effects of certain known antifilarials on the immune response of *M. natalensis* infected with *Brugia malayi*, *Indian J med Res*, **83** (1986) 155-61.
3. Sen A B, Chatterjee R K & Bhattacharya B K, Studies on the filarial infection in the Indian jungle crow, *Corvus macrorhynchos* Wagler, *Ann trop Med Parasit*, **59** (1965) 346.
4. Sen A B & Chatterjee R K, Effect of different stimuli on the migration of *Chandlerella hawkingi* microfilariae in naturally infected jungle crow, *Indian J Parasit*, **1** (1977) 47.
5. Saxena J K, Bose S K, Chatterjee R K, Ghatak S & Sen A B, Occurrence and concentration of biogenic amines in microfilariae and adults of *L. carinii*, *Exp Parasit (New York)*, **43** (1977) 239.
6. Singh D P, Rathore S, Misra S, Chatterjee R K, Ghatak S & Sen A B, Studies on the causation of adverse reactions in microfilaraemic host following diethylcarbamazine therapy (*Dipetalonema vitae*) in *Mastomys natalensis*, *Trop Med Parasit (West Germany)*, **36**(1) (1985) 21-24.
7. Chandra R, Govil Promila, Chandra S, Katiyar J C & Sen A B, *Wuchereria bancrofti* larval antigen in the diagnosis of human filariasis by skin test, *Indian J med Res*, **62** (1974) 1017.
8. Chandra R, Katiyar J C, Murthy P K, George P A & Sen A B, *Indian J med Res*, **68** (1978) 61.
9. Sen A B & Hawking F, Screening of cesticidal compounds on a tapeworm *Hymenolopis nana* in vitro, *Br J Pharmacol*, **15** (1960) 436.
10. Hawking F & Sen A B, The trypanocidal action of homidium, quinapyramine and suramin, *Br J Pharmacol*, **15** (1960) 567.
11. Bhattacharya B K & Sen A B, A rapid method for the screening of compounds in experimental protozoal infections, *Arch int Pharmacodyn*, **137** (1962) 61.
12. Sen A B, Katiyar J C & Bhattacharya B K, Cesticidal activity of substituted dihydrobenzo-1,3-oxazines, *Nature, Lond*, **207** (1970) 1259.
13. Khare S, Saxena J K, Sen A B & Ghatak S, Erythrocyte membrane-bound enzymes in *Mastomys natalensis* during *Plasmodium bergeri* infection, *Aust J exp Biol med Sci*, **62** (1984) 137-43.
14. Guru P Y & Sen A B, In vitro cultivation of erythrocytic stages of *Plasmodium bergeri*, *Indian J Parasit*, **2**(2) (1978) 185.
15. Bhattacharya B K, Sen A B & Talwalkar V R, Pharmacologically active substances in the plasma of guineapig infected with *Trypanosoma evansi*, *Arch int Pharmacodyn*, **156** (1965) 106.

## H G Sen

The importance of developing a laboratory-adapted human hookworm strain has been recognized for decades all over the world. However, attempts to grow these parasites in laboratory animals were unsuccessful, since the host did not provide a medium suitable to hookworms, which were finally rejected long before they developed to maturity. Research on hookworm disease in the laboratory has not been feasible. We have no clear knowledge how the immune mechanisms operate in man and about the damage caused by hookworm parasites, particularly in endemic areas. The success of developing an ideal therapeutic drug for man is a far-off goal in the absence of a proper experimental model, suitable for studies in the laboratory.

Sen and Seth<sup>1</sup> for the first time reported the complete development of human hookworm, *N. americanus*, in golden hamsters, *Mesocricetus auratus*. These animals when infected neonatally provide favourable media for these parasites, which survive to maturity in contrast to older hamsters which expel the entire parasite load while they are immature. Even though these parasites developed to maturity in hamsters infected at birth, serial passage of the hookworm larvae into hamsters was extremely difficult if not impossible because of the low yield of

infective larvae from faecal cultures. Administration of corticosteroid during serial passage, however, increased the susceptibility of hamsters to infection. The first five serial passages were done under the influence of a low dose of corticosteroid (1.3 mg/kg/day) at 21 days post-infection, after which the steroid was never administered. Adaptation of these parasites to the hamster occurred from the sixth serial passage and thereafter following the complete cessation of steroid<sup>2</sup>. Adaptation of hookworms to the hamster, an abnormal host, was manifested by increased clinical symptoms, e.g. anaemia, hypoproteinaemia, etc., the egg laying pattern and the greater worm recovery. Termination of infection is manifested through spontaneous expulsion of mature parasites or death of the animal in several weeks or months. The parasites not only survive in the hamster but also reach normal size comparable to that found in man, mature, mate and produce viable eggs, which on culturing give rise to infective larvae. It is reasonable to believe that this host provides a favourable medium and satisfies the life requirements of this adapted population. This new host-parasite system serves as an excellent tool for the study of experimental human hookworm infections, since a relatively high percentage of infective larvae reach sexual maturity and

---

Chief, Research and Development, Natural Products Division, Dey's Medical Laboratories, 62, Bandal Road, Calcutta-700019; Residence : AC91, Sector 1, Salt Lake, Calcutta-700064.

The work covered here was done at CIBA-Geigy Research Centre, Bombay, during the period 1962-80.



establish as a stable population, which is compatible to the hamster. The human hookworm-hamster model provides a valuable model for various investigations, which have hitherto not been possible. It opens avenues for the discovery of new anthelmintics in contrast to the rodent or poultry gastro-intestinal nematodes currently used as test organisms.

### Experimental Chemotherapy

In the course of a routine anthelmintic screening programme, among several thousand compounds tested in this new host-parasite model, 4-isothiocyanato-4'-nitrodiphenylamine (C 9333-Go/CGP 4540; generic name amoscanate) has been found to be highly active against *N. americanus* at a single dose of 10 mg/kg with 100% elimination of parasites in patent infection. This drug has also shown a very high therapeutic value (almost 100%) when tested clinically in man not only against both the species of hookworms but also against *Ascaris lumbricoides*, *Trichuris trichiura* and *Enterobius vermicularis* at single or multiple doses<sup>3-5</sup>. The therapeutic efficacy of amoscanate in man thus proves beyond doubt the validity of this model. There is every reason to believe that this drug will be extremely useful for mass therapy and will be the drug of choice, particularly for the treatment of hookworm disease in man all over the world.

### Pathological Studies

Even though hookworm anaemia in man is common in all tropical countries, information about the nature of anaemia is inadequate. Loss of iron has been demonstrated as the main cause of anaemia, which is directly related to the worm load in patients on substandard diet. It has been claimed that a single

hookworm parasite (*Necator*) removes as much as 0.50 ml of blood per day [Faust EC & Russell PP, Craig and Faust's *clinical parasitology* (Lea and Febiger, Philadelphia) 3rd Edn, 1964, 383]. Some of the clinical symptoms besides anaemia have been attributed to the effects of toxin (?) liberated by the parasites. Till now no such toxin has been isolated. The hamster-hookworm model will doubtless provide a powerful system for radioactive studies with <sup>51</sup>Cr or <sup>51</sup>Fe and will reveal the host-parasite association resulting in blood loss and other damages.

### Immunological Studies

We have no clear knowledge about the patient's resistance in the production of hookworm disease. It is known that man does not develop complete immunity to hookworms and people exposed to infection continue to carry worms for many years if not all their lives [Ball FAJ & Bartlet A, *Trans R Soc trop Med Hyg*, **63** (1969) 362-69]. Epidemiological studies conducted under the sponsorship of the World Health Organization indicate that most patients who suffer from severe hookworm disease are poorly nourished and, therefore, poorly supplied with the mechanism of resistance to the infection. In many areas, man is constantly exposed and re-exposed to severe infections from his heavily contaminated environment; yet in these circumstances, he survives. Despite the many decades of investigations on hookworm, we know almost nothing as to how he gets along with the parasite living under endemic conditions.

### Diagnosis

Diagnosis of hookworm infection in man by skin hypersensitivity test has been

reported by Lobel *et al.* [*Am J Epidem*, **87**(1) (1968) 58-72] and others. We do not have enough data about the validity of this test method in view of non-specific reactions. In the absence of clear evidence, this may not be an acceptable method for the diagnosis of hookworm infection in both endemic and nonendemic areas.

### Biochemical Studies

Nothing is known about the nutritional requirements of adult human hookworms. All we know is that the parasite while attached to the mucosal layer of the small intestine sucks blood, which passes through its own digestive tract without disintegrating erythrocytes. The plasma along with the rbc is excreted through its anal pore. It is very likely that glucose, albumin, amino acids or other essential metabolites alone or in combination are absorbed by epithelial cells of the gut of parasites for their development and reproduction. Studies on metabolic pathways of human hookworms have never been attempted due to the lack of adult specimens, which are limited to human hosts. According to the new host-parasite system developed by us, any desired number of both mature and immature hookworm parasites are easily obtained from hamsters at autopsy and can be used for various biochemical explorations.

The above studies in the human hookworm hamster model need to be undertaken; the results obtained from such investigations will furnish valuable guidelines in understanding the association between man and his hookworm parasites.

In conclusion, adaptation of the human hookworm, *Necator americanus*, to

golden hamsters, *Mesocricetus auratus*, is a significant advance in tropical medicine, because at long last the hookworm disease can be studied in the laboratory. The parasites not only survive in the hamster, an abnormal host, but are also established and maintained independently. They reach normal size comparable to that in man, mature, mate and produce viable offsprings. This new host-parasite system serves as an excellent tool in the study of experimental human hookworm infections by overcoming the severe limitations imposed by specificity to human hosts. It is expected that this hamster hookworm model will provide a satisfactory solution to the problem of hookworm disease in man either by the discovery of a more potent but less toxic drug or by the production of a vaccine of high protective value against the infective larvae. Stoll [*Exp Parasit*, **12**(4) (1962) 241-52], who had studied hookworm infections throughout the world for almost half a century, stated that "As it was when I first saw it, so it is now, one of the most evil of infections. Not with dramatic pathology as are filariasis, or schistosomiasis, but with damage silent and insidious. Now that malaria is being pushed back, hookworm remains the great infection of mankind. In my view it outranks all other worm infections of man combined with the possible exception of ascariasis, in its production frequently unrealized of human misery, debility and inefficiency in man in the tropics".

### Selected Publications

1. Sen H G & Seth D, Complete development of the human hookworm, *Necator americanus* in golden hamsters, *Mesocricetus auratus*, *Nature*, **Lond**, **214** (1967) 609-10.
2. Sen H G, *Necator americanus* : Behaviour in hamsters, *Exp Parasitol*, **32**(1) (1972) 26-32.



3. Vaidya A B, Sen H G, Mankodi N A, Paul T & Sheth U K, Phase I tolerability and searching dose studies with 4-isothiocyanato-4-nitrodiphenylamine (C 9333-Go/CGP 4540)—A new anthelmintic, *Br J clin Pharmac*, **4** (1977) 463-67.
4. Doshi J, Vaidya A B, Sen H G, Mankodi N A, Nair C N & Grewal R S, Early dose searching of a new anthelmintic 4-isothiocyanato-4-nitrodiphenylamine (C 9333-Go/CGP 4540) for the treatment of hookworm infection, *Am J trop Med Hyg* **26**(4) (1977) 636-39.
5. Vakil B J, Dalal N J, Shah P N, Mankodi B A, Sen H G & Vaidya A B, Clinical evaluation of a new anthelmintic C 9333 Go/CGP 4540 in human hookworm infection, *Trans R Soc trop Med Hyg*, **71**(3) (1977) 247-50.

## P K Sen

From the beginning of his postgraduate work in 1932, Sen has been continuously engaged in research on tuberculosis and chest diseases. He has published about 100 papers and has contributed chapters on tuberculosis in several important books<sup>1-4</sup>. He was closely associated with the planning and execution of several national studies like the 'National Sample Survey of Tuberculosis'<sup>5</sup> and 'Resistant TB infection in the community'<sup>6,7</sup>, while serving as the Chairman of Tuberculosis Sub-committee and Expert Group of the Indian Council of Medical Research, and Technical Committee of the Tuberculosis Association of India. Sen's studies covered wide areas in pathology, bacteriology, epidemiology and biochemistry but they had mainly a clinical bias. A brief review of his major contributions is given below.

### (1) Nutrition in Tuberculosis<sup>8</sup>

Work in this area was done at the Reinickendorf Hospital, University of Berlin, at a time when notions about extra and special diet for tuberculosis were being advocated everywhere. Studies in man, both clinical and experimental, showed that extra and special diet is not necessary. The validity of this has now been proved through chemotherapeutic work.

### (2) Tuberculosis and Silicosis in Coal Miners<sup>9</sup>

(i) Work done on this problem at the Department of Tuberculosis, University of Wales, contradicted the earlier view that coal miners in Wales suffer much less from tuberculosis than the civilian population. This work, including histological, bacteriological, clinical and X-ray investigations, showed that the miners suffer much more than the others. The results of this study proved useful in respect of programme of tuberculosis control in this important industrial coalmine area.

(ii) A section of coalminers develop 'silicosis' with gradually increasing dyspnoea till they become incapacitated in about two years. It was believed that gradual extension of silicotic lesions occurs even if the miner leaves the job early. So, they worked till they were completely disabled. This study showed that the real cause of severe shortness of breath is "emphysema" and not so much the developing fibrosis in the silicotics. Such dyspnoea can be prevented significantly if the miner is shifted to a surface job from the underground work at the start of shortness of breath. This knowledge led to amelioration of the sufferings of a large number of coalminers.



(iii) It was held that all silicotics die of tuberculosis. Sen's study showed this to be untrue, though there is heavy incidence of tuberculosis among them.

### **(3) Mapping Tuberculosis in West Bengal<sup>10,11</sup>**

This study was conducted at the All India Institute of Hygiene and Public Health, Calcutta, under the sponsorship of the Indian Council of Medical Research with the objective of assessing the status of this disease in different categories of areas like industrial, urban and rural. The survey was begun with tuberculosis and chest diseases in the jute industry and was extended to tuberculosis in different areas. It indicated for the first time how the disease differs from one type of locality to another and how it spreads from the industrial to rural areas. The knowledge gained proved useful in the designing of control programmes. A 'National Sample Survey', roughly comparable to this survey conducted two decades later, seemed to indicate that the disease had spread quickly in the rural areas from 0.5% to 1.5% of inhabitants and remained almost static in urban area; now tuberculosis is equitably distributed in the rural and urban areas and may be regarded as an endemic disease. This finding is of value in the formulation of control programmes.

### **(4) Domiciliary Treatment of Pulmonary Tuberculosis<sup>12-15</sup>**

This work, done at the Chest Department, Medical College and All India Institute of Hygiene and Public Health, was one of the pioneering studies showing that 'home treatment' can be made as effective as that at the hospital. Today, this concept has become the backbone of the tuberculosis control programme in India.

Following this work, Sen made several studies on various aspects of domiciliary treatment aimed at improvement of practices for this measure, including its long-term impact in the rural areas, home contacts, etc.

### **(5) Prevalence of 'Drug-Resistant' Infection in Tuberculosis Cases<sup>16,17</sup>**

Work on this aspect carried out at B C Roy Tuberculosis and Chest Diseases Research Institute, Calcutta, revealed that development of resistant TB bacillary strains during the course of anti-TB chemotherapy is very dangerous for both the patient and the public. Besides the ICMR sponsored study (with Sen as chairman of the working group of three), he made a number of studies to show how this unwanted phenomenon develops in the patients through irregular drug intake with respect to time; he also suggested some preventive measures.

### **(6) Planning Anti-TB Chemotherapy from the Biological Values of Drugs<sup>18-22</sup>**

The aim of this work, done at B C Roy Tuberculosis and Chest Diseases Research Institute, was to find the most effective drug combinations against tuberculosis. Innumerable combinations were tried in field surveys for about 30 years. They are still continuing.

To overcome the problem of reducing the number of field trials, Sen assayed several anti-TB drugs in respect of the following points: Their highest blood concentrations; time when they reach the peak; time for which they retain anti-bacterial action; the manner in which they cover each other to prevent emergence of resistant strains, etc. On the basis of the results obtained he planned drug regimen

for highest anti-TB action from the biological values alone. The drug regimen suggested on the basis of these considerations appears to be similar to those suggested by field trials in several other places. He had, therefore, suggested that on the introduction of any 'new drug' in the field, these values should be assessed first and, if necessary, a few pilot field trials should be made prior to acceptance at a wider scale. To him it seems a reasonable deduction. If accepted by others, the benefit will be very great both technically and financially.

### (7) Anti-TB Drugs during Pregnancy

Opinions about harmfulness of these drugs to the foetus in the womb differ. So, an accepted common stream of action cannot be designed for chemotherapy of such mothers. This study attempts to solve this problem partly through followup of children of the same mothers, so exposed, pre- and post-born in regard to their 'health'. In this initial study, no harm could be detected in the exposed children as compared to the others<sup>23</sup>.

### Selected Publications

1. Sen P K, in *Text Book of Tuberculosis (Chemotherapy)* (Vikas Publishing House/ Tuberculosis Association of India) 1972.
2. Sen P K, *A textbook of preventive and social medicine* (Indian Public Health Association & Allied Agency, Calcutta) 1971.
3. Sen P K, Tuberculosis, in *A treatise on hygiene and public health* by B N Ghosh, edited by J K Bhattacharya (Scientific Publishing Co) 15th Edn, 1969, 584.
4. Sen P K, *Respiratory diseases*, IMAS special publication series No. 6 (Indian Med Association) 1982.
5. Sen P K, in *National Sample Survey of Tuberculosis in India 1955-58* (ICMR, New Delhi) 1959.
6. Sen P K, Prevalence of drug resistance in patients with pulmonary tuberculosis presenting for the first time with symptoms, Part I, *Indian J med Res*, **56** (1968) 1617-30.
7. Sen P K, Prevalence of drug resistance among all patients in urban clinics, Part II, *Indian J med Res*, **57** (1969) 823-35.
8. Sen P K, Ernahrungsgesfragen bei Lungen Tuberculose Untorbesonderer berucksichtigung der vitamine and des fottes (Problems of nutrition in pulmonary tuberculosis with special reference to vitamins and fats), *Doctorate Thesis, University of Berlin (Duch and Ceriagadruckere Hans Heeneman)* 1933.
9. Sen P K, Tuberculosis and silicosis in South Wales coalminers, *J ind Hyg, Toxicol*, **19** (1937) 225.
10. Ukil A C & Sen P K, Tuberculosis survey of industrial population in the neighbourhood of Calcutta, *Annual Report, Indian Council of Medical Research*, 1952.
11. Ukil A C & Sen P K, Tuberculosis in relation to jute industry, *Proc Indian Sci Congr*, (1940).
12. Sen P K, *Doemiliary treatment in tuberculosis*, Paper presented at the International Union against Tuberculosis Committee Meeting at Paris, July 1958.
13. Sen P K, Domiciliary treatment of pulmonary tuberculosis, Record of 5,833 cases, *Indian J Tuberc*, **7**(3) (1960) 76-84.
14. Sen P K & Garai R, Impact of tuberculosis control programme in rural areas, *Indian J Tuberc*, **20**(3) (1973) 125-29.
15. Sen P K & Sil A K, Influence of type and time of contact in pulmonary tuberculosis, *Proc 20th Tuberculosis and Chest Diseases Conference*, February 1965.
16. Sen P K & Roy B N, Bacillary resistance to drugs in tuberculosis, A trend over 13 years, *Indian J Tuberc*, **22**(2) (1975) 68-72.
17. Sen P K, Disease, drugs and domiciliary treatment in tuberculosis, *RV Rajam Memorial Oration Ann natn Acad med Sci (India)*, **17**(3) (1981) 109-26.
18. Sen P K, Anti-TB drug regimen (An approach through pharmaco-therapeutic basis), *Indian J Tuberc*, **28**(4) (1981) 191-99.
19. Sen P K, Planning chemotherapy against tuberculosis, *Indian J Chest Dis*, **24** (2,3) (1982) 203-7.
20. Sen P K, Biological values of anti-TB drug action, *Proc Eastern Regional Tuberculosis Conference*



- of International Union against Tuberculosis, Jakarta-Indonesia, November 1983, 26.*
21. Sen P K, Isoniazid inactivation in tuberculous patients, *Indian J med Res*, **60** (1972) 19-27.
22. Sen P K, Rifampicin: Biological values for action and intolerance, *Indian J Tuberc*, **32** (1985) 81-85.
23. Sen P K, Anti-TB drugs during pregnancy, *Summary Trans, 14th Eastern Regional Tuberculosis Conference of the International Union against Tuberculosis, Kathmandu, Nepal, 24-29 November 1985, 85.*

## K N Sharma

Original contributions of fundamental importance have been made by Sharma in the field of neural control systems for ingestive behaviour and energy homeostasis. The elucidation of hypothalamic-limbic and alimentary receptor mechanisms has led to conceptional changes with considerable significance in applied nutrition and ecology of hunger. It is now well recognized that chemosensory signals from food not only provide the sensory basis of the hedonic metrix, but the sensory and nutrient signals also act as primary biological variables in food acceptability, choice and intake. In this process, the brain modulates intake by continuous comparison of shortage or surplus in the body energy-nutrient pool with sensory and nutrient signals in the available diet.

While under energy surfeit conditions metabolic signals predominate, in chronic energy deficient conditions sensory signals become prepotent. This biasing of the signalization pattern is operative at local, gastro-intestinal, systemic and several levels of the neuraxis, and allows filtering of the information right at the peripheral site. This, in turn, leads to the appropriate behavioural and metabolic responses by the organism in strict accordance with the internal (body) and external (environmental) requirements.

Extension of these animal experimental models to studies on human nutrition involving socio-economic, anthropometric, clinico-metabolic and cross-cultural attributes has led to newer directions of approach in the understanding of food habits, food preferences, and food optimization. This work has received global attention.

A pioneering effort has been the publication by MIT Press, USA, of a monumental work on '*Canine brain in stereo-taxic co-ordinates*'. The first of its kind from this country, it has potential implications in experimental neurology and neurosurgery.

In the realm of classical neurophysiology, it has been shown for the first time that dual differential centrifugal control of visceral receptors exists; it forms an important base for explaining visceromotor behaviour.

In recent years, attention has been focussed on the spino-peripheral organization of nociception and it has been demonstrated that the interaction between low threshold mechanoreceptors and 'C' fibre nociceptive afferents may take place, among other sites, at the peripheral locus.

The future plan of work envisages investigative models for nociception as a feature in the broad spectrum of other sensory cues linked to genetic,



physiologic, ethnic, cross-cultural, socio-economic and environmental factors.

### Selected Publications

1. Sharma K N, Anand B K, Dua S & Singh B, Role of stomach in the regulation of activities of the hypothalamic feeding centres, *Am J Physiol*, **201** (1961) 593.
2. Sharma K N & Nasset E S, Electrical activity in mesenteric nerves after perfusion of the gut lumen, *Am J Physiol*, **202** (1962) 725.
3. Sharma K N, Anand B K & Dua-Sharma S, Effect of hypothalamic lesions on experimental gastric ulceration in rats, *Indian J med Res*, **51** (1963) 708.
4. Sharma K N, Receptor mechanisms in the alimentary tract: Their excitation and functions, in *Handbook of physiology*, Sect 6, *Alimentary canal*, Vol I (American Physiological Society, Washington) 1967, 225-37.
5. Sharma K N, Alimentary receptors and food intake regulation, in *Chemical senses and nutritive processes*, edited by M Kare, O Maller (Johns Hopkins Press, Baltimore) 1967, 281-91.
6. Jacobs H L & Sharma K N, Taste versus calories: Sensory and metabolic signals in the regulation of food intake, *Ann N Y Acad Sci*, **157** (1969) 1084.
7. Sharma K N, Jacobs H L, Gopal V & Dua-Sharma S, Vago-sympathetic modulation of gastric mechanoreceptors: Effect of distension and nutritional state, *J Neural Trans*, **33** (1972) 113.
8. Sharma K N, Ontogenetic and nutritional modulation of alimentary signalization, in *Genetic, nutritional and environmental factors in brain growth and development*, edited by M A Brazier (Raven Press, New York) 1975, 191-202.
9. Sharma K N, Dua-Sharma S & Jacobs H L, Electrophysiological monitoring of multilevel signals related to food intake, in *Neural integration of physiological mechanisms and behaviour*, edited by G Mogenson & F Calaresu (University of Toronto Press, Toronto) 1975, 194-212.
10. Moskowitz H W, Kumaraiah V, Sharma K N, Jacobs H L & Dua-Sharma S, Cross-cultural differences in simple taste preferences, *Science*, **190** (1975) 1217.
11. Moskowitz H R, Kumaraiah V, Sharma K N, Jacobs H L & Dua-Sharma S, Effects of hunger, satiety and glucose load upon taste intensity and taste hedonics, *Physiol Behav*, **16** (1976) 471.
12. Sharma K N, Dua-Sharma S, Rao B S & Jacobs H L, Neural plasticity and hedonic matrix: Relevance of animal models to human nutrition and food preferences, in *Neural growth and differentiation*, edited by E Meisami & MAB Brazier (Raven Press, New York) 1979, 351-64.
13. Sharma K N, Nijima A & Dua-Sharma S, Gastric modulation of gustatory evoked pancreatic vagal efferent activity, *Proc Int Cong Physiol Sci*, Vol 14, 1980, 696.
14. Sharma K N, Alimentary signalization and homeostatic motivation, *First Indo-Soviet Symposium on Neuro-physiology*, Tzahkadozor, 11-13 October 1984.
15. Sharma K N, Igestive behaviour and neural encoding: Systems approach, *Pragmatic Cybernetics*, edited by A Ghoshal (South Asian Publ., New Delhi), 1986, 85-95.
16. Radhakrishnan V & Sharma K N, Effect of selective gastric vagotomy on gustatory behaviour in rats, *J Auton Nerv Syst*, **15** (1986) in press.

## A K P Sinha

A problem of tremendous importance today is that of comprehending the diverse facets of political development and of influencing the dynamic processes of nation-building, for which political consensus has to be developed. The problem of creating political consensus in most of the developing countries is, in part, one of building new and more universal means of national communication, of establishing more effective channels of communication and transportation, so that all segments of the society could become involved with one another more closely. In part, this problem is also related to the attitudes and sentiments of the individuals which emerge out of their cumulative communication experiences. Obviously, it is a problem for the adequate solution of which we need scientific knowledge based on well-tested hypotheses. Sinha's major contributions in some of these areas are discussed below in brief.

### Experimental Psychology

In terms of the emerging new challenges, the experimental psychologists today do not have to contribute merely to finding solutions for long-standing problems relating to transfer effects<sup>1,2</sup>, Mueller Lyer illusion<sup>3</sup> and the like. They have also to think of research problems such as those involved in the man-in-space

programmes. Viewed in this light, brain dynamics is a very productive area of investigation. Adequately controlled studies have to be conducted to find out where information is stored in the brain, how it is stored there and what influences its storage. Another fruitful area for research is the one related to the decision-making process. In our society, we have a host of problems concerning the rural population demanding urgent solution based on the findings of experimental studies. As Sinha<sup>4</sup> has stated, "In a rapidly changing and interrelated world, we cannot afford the luxury of trial and error in dealing with problems which are of vital concern to our people and country. It is our responsibility as psychologists to remind those engaged in developing sophisticated procedures for coping with problems like nuclear power and natural hazard management that the importance of the human component in implementing the techniques should not be lost sight of". We have yet to go a long way for making the outcome of experimental research useful to the society. In terms of the high visual acuity of hawks, a thinking seems to be growing as to how they could be used for rescuing persons lost in wilderness. Animal studies are needed with a view to determining the possible use of animals to perform tasks commonly done by either men or machines.

---

Formerly, Senior Professor & Director, Institute of Psychological Research & Service, Patna University, Patna; Residence : Road No. 3, Rajendra Nagar, Patna-800016.



## Psycholinguistics

Although human societies all over the world have language, the actual form of language usage varies from one population to another. And so, it becomes necessary to study the problems of language in the context of the diverse social processes in operation in different societies and cultures. One broad area in which research is very much needed may be designated as language information and communication. A longitudinal study of cognitive and social determinants of language acquisition could perhaps be fruitfully undertaken. Experiments that require individuals to integrate pieces of incoming information and deduce appropriate conclusions therefrom open new vistas of research on the role of presuppositions and expectations in linguistic inferences. Systematic researches in semantic memory research may advance our knowledge on the process of retrieving semantic information, which serves the function of a rather fundamental process in almost any kind of cognitive behaviour. A good deal of attention is required to be given to the study of gestures, linguistic variants and paralinguistics. Apart from the biological, sociological and anthropological issues involved in psycholinguistics, there are clinical problems arising from disorders of speech and language that seem to be no less potent and significant. Psychologists and psychiatrists have tried to interpret the language of schizophrenics which presents a clinical picture very different from aphasia. But it still remains a challenging problem for the psycholinguists to deal with by undertaking research studies in this field<sup>5</sup>.

## Guidance

The increasing educational opportunities of the present century, the wide range of possible occupations offered by our complex society, the rapidity with which technological advances are taking place, emergence of tensions of different kinds in both national and international spheres, and the accelerated rate of demands on the developmental capacities of individuals have created a situation in which assistance has to be rendered to young men and women to set and maintain a personal pace that is adjusted to the changing pace in society. They have to be helped to find meaning in the present and to see the relation of the present with the past and the future. All these clearly show the need for guidance and sophisticated studies in this area. The need for studying the problems of the adolescent, the aged, and the physically and mentally handicapped should be rather on a priority basis. Much research has to be conducted on the nature of student unrest, activism and on the patterns of their development. It would also be worth while conducting research with a view to ascertaining whether or not there are distinctive personality patterns for attaining success in certain occupations<sup>6</sup>. In order to be able to render proper guidance to the child, the current need is to formulate suitable hypotheses and to evolve fruitful strategies of research, also keeping in view the demands of the rural sector. Studies have to be conducted on family structure, parent-child interaction, caste awareness and attitudes, moral development and social control, and cognitive style of children in rural and urban families<sup>7</sup>.

## Social Problems

Human society, as one can easily discern, is in a state of disharmony and

discord, and perhaps the only hope for man is to turn the light of his intellect against the problems of conflict in society. A society will not survive unless its individuals are motivated to serve its varied needs. The problems of poverty and dependency, crime and delinquency, sickness and physical handicap, unemployment and discontent, ignorance and superstition, war, population pressure, prejudice, social distance and stereotypes often mar the prosperity and progress of the society. Most of these problems are rooted in the social maladjustment caused by social change. A central problem, therefore, is that of adjusting our social life and our social institutions, so that as individuals and as communities we may enjoy the fruits of civilization and promote further progress. The Indian society, like all other developing societies, is changing at a rapid pace. It poses a challenge that calls for the mobilization of the available resources for the betterment of the country and its all round development. Psychological research, therefore, should be conducted with a view to determining the psychological variables involved in any programme of social change. We must have useful tested knowledge which may be brought to bear significantly on the society's most urgent problems. Research will, therefore, have to be conducted, keeping in view the needs and cognitive functioning of the group of persons involved in the process of any programme of change, so that the findings of the same could be utilized fruitfully. Psychological research should be directed towards finding out those specific psychological processes and variables that intervene between poverty and behaviour<sup>8</sup>. The findings of studies on stereotypes<sup>9,10</sup>, social distance<sup>11</sup>, prestige-suggestion<sup>12</sup>, and on

the relationship between ethnic stereotypes and social distance<sup>13</sup> clearly demonstrate their utility in the formulation of programmes of both national and international significance.

### A Research Strategy

Social psychologists have often overemphasized the research design employed in the traditional laboratory deception study in order to ensure precise control over relevant variables with a view to making valid causal inferences as also to demonstrate scientific respectability. But in terms of the ever-increasing demands upon social psychologists for relevant studies which can offer adequate solutions to diverse social problems, it would be advisable to test appropriate hypotheses under both laboratory and natural conditions in order to be able to make precise statements about causal relationships and to specify the relative strength and the generality of the results obtained. If social psychologists acquire a more broad and more flexible orientation towards research, both the theoretical scope and the social relevance of the field should increase significantly. Further, as biological and biochemical techniques develop beyond their present level of sophistication and refinement, they will have ever greater applicability to research in experimental social psychology. We need better theories of social behaviour. Research dealing with single conceptual levels of analysis may perhaps provide the basis for a synthesis of concepts derived from many levels<sup>14,15</sup>.

### Selected Publications

1. Sinha A K P & Sinha S N, Intersensory transfer in learning sequences, *J exp Psychol*, **60** (1960) 180-82.



2. Sinha A K P & Prasad M B, Transfer of training as a function of the method of first-task learning, *J gen Psychol*, **66** (1962) 311-14.
3. Sinha A K P & Sinha S N, Mueller Lyer illusion in subjects high and low in anxiety, *Perceptual and Motor Skills*, **24** (1967) 194.
4. Sinha A K P, Trends in experimental psychology, *Indian J Psychol*, **54** (1979) 1-7.
5. Sinha A K P, Frontiers of research in psycholinguistics, *Psycholinguia*, **10**(2) (1980) 1-7.
6. Sinha A K P, Humanization of technological society, *Third Jamuna Prasad Memorial Lecture*, 1982.
7. Sinha A K P, Understanding the child, *Indian J Psychol*, **54** (1979) 97-104.
8. Sinha A K P, The role of psychologist in a developing society, *Inaugural Address, Golden Jubilee Celebrations, Indian Psychological Association*, 1976.
9. Sinha A K P & Upadhyay O P, Stereotypes of male and female university students in India towards different ethnic groups, *J Social Psychol*, **51** (1960) 93-102.
10. Sinha A K P & Upadhyay O P, Change and persistence in the stereotypes of university students towards different ethnic groups during Sino-Indian border dispute, *J Social Psychol*, **52** (1960) 31-39.
11. Sinha A K P & Upadhyay O P, Eleven ethnic groups on a social distance scale, *J Social Psychol*, **57** (1962) 49-54.
12. Sinha A K P & Ojha H, An experimental study of the operation of prestige-suggestion in extroverts and introverts, *J Social Psychol*, **61** (1963) 29-34.
13. Sinha A K P, Relationship between ethnic stereotypes and social distance, *Psychol Rep*, **28**(1) (1971) 216.
14. Sinha A K P, Psychology and social change, *Inaugural Address, Regional Convention of International Council of Psychologists*, 1981.
15. Sinha A K P, Guidance at various educational levels, *Indian J Psychom Educ*, **13** (1982) 1-10.

## S Sriramachari

Sriramachari, currently Additional Director-General, Indian Council of Medical Research, New Delhi, has been a full-time research worker of the Indian Council of Medical Research for more than 35 years. He is also the founder-Director of the Institute of Pathology-ICMR (formerly known as the Indian Registry of Pathology).

The scientific contributions of Sriramachari cover a wide spectrum of original investigative work in the following broad fields: (1) Nutritional Pathology; (2) Histochemistry; (3) Osteopathology; (4) Hepatic Pathology; (5) Medical Educational Technology; (6) Neuropathology; and (7) Pathological and Toxicological Aspects of Bhopal Gas Disaster.

### (1) Nutritional Pathology

#### (i) *Nutritional liver injury*

He started as a Lady Tata Research Scholar under late Prof M D Ananthachari and was among the earliest workers to carry out liver biopsy studies in India. In 1950-51, he studied the correlation of biochemical and histopathological changes in adults suffering from malnutrition in comparison with those with cirrhosis<sup>1,2</sup>.

He joined the Nutrition Research Laboratories, Coonoor, as an Assistant Research Officer, in which capacity he worked up to 1951-59. Both in association

with Prof. V Ramalingaswami and soon after independently, he published a series of scientific papers on a wide range/variety of pathology of nutritional disorders.

The papers on pathology of kwashiorkor are among the first reports from India<sup>3,4</sup>. With the corresponding experimental studies in animals subjected to protein or combined calorie deficiency, he differentiated the picture of the fatty liver on the one hand and a marasmic liver on the other<sup>5,6</sup>. This work is of great importance even today in the understanding of protein energy malnutrition. One of the most important observations of Sriramachari is based on an 8-year follow-up study with the help of liver biopsy of kwashiorkor that none of the cases ended up with hepatic fibrosis nor progress to cirrhosis<sup>7</sup>. This was an important piece of information right from that time.

Subsequently, on his own, he carried out an extensive study on different types of fatty livers caused by protein and lipotrope deficiency and obtained histopathological evidence for periportal or centrilobular changes in the liver in one and the same species of animal, viz., the rat. This has a great bearing on the pathogenesis of fatty livers<sup>8</sup> of kwashiorkor.

Sriramachari participated in some of the crucial experiments of late Dr Patwardhan



and late Dr Phansalkar involving clinical and experimental work on the beneficial effects of diets based on mixtures of vegetable protein, which established the scientific rationale that suitable combinations of plant foodstuffs are nowhere inferior to animal protein<sup>9</sup>. This work has far-reaching nutritional and agronomical implications.

He further studied the long-term sequelae of such fatty livers and established the fact that under the influence of continuing protein deficiency, even if hepatic fibrosis or cirrhosis developed, the same effects are not sustained due to the phenomenon of reversibility of cirrhosis of even dietary origin<sup>10</sup>. Through a well planned series of toxic liver injuries induced by carbon tetrachloride<sup>11</sup>, he established the theory of reversibility of both dietary and toxic experimental cirrhosis as a result of concurrent protein deficiency. Thereby he was able to explain certain anomalous situations observed in clinical practice. Thus, children with kwashiorkor never progress to cirrhosis<sup>12</sup> unlike the well-fed children ending up with relevance of the hepatic fibrosis of Indian childhood cirrhosis.

This broad area of work was not only the theme of his DSc thesis, but also received recognition from renowned authorities like Sir Roy Cameron. The significance of these findings was explained in a subsequent paper<sup>13</sup> at the instance of Dr V R Khanolkar, a doyen among Indian pathologists. It was also published in *Collagen*, edited by N Ramanathan (John Wiley and Sons, New York, 1962).

#### (ii) *Other Nutritional Disorders*

Sriramachari made significant contributions in other fields of nutritional

pathology. He had shown that in addition to the liver, other organs, such as aorta and the cardiovascular system and the endocrines, are severely affected in experimental protein deficiency<sup>14</sup>. Later, this information was confirmed by other nutritional workers in the country.

His studies in nutritional pathology of vitamin A deficiency carried out along with Prof. Ramalingaswami and Dr Leach of Oxford University in the early fifties provided anatomical basis for the retinal involvement in vitamin A. Subsequently, in collaboration with Prof. J Ganguly and Dr Juneja, he studied the differential effects of the alcohol, aldehyde and acid forms of vitamin A on the male and female gonads of the rat. It was shown that while retinol had an all round protective effect, retinoic acid, in fact, resulted in gonadal damages.

### (2) **Histochemistry**

Even as a Lady Tata Research Scholar, Sriramachari was perhaps the first medical scientist to apply the principles of histochemistry based on his work on carbowaxes<sup>15</sup>. Subsequently, he used these techniques and several other enzyme histochemical techniques in the study of osteopathology (mode of action of vitamin D) and tetracycline labelling of newly formed bone, neuromuscular disorders and experimental brain oedema.

### (3) **Osteopathology**

In the field of osteopathology, Sriramachari has made notable contributions in two fields. The first one pertains to a series of papers based on exhaustive histochemical studies on the mode of action of vitamin D and the sequences of mineralization of the rachitic epiphyseal cartilage under the influence of vitamin D and starvation. The early

demonstration of non-calcific phase as a result of glycogenolysis of chondrocytes immediately followed by the calcific phase is of basic significance.

In recent years, Sriramachari has been involved in a new approach towards the crystalloid-colloid interaction of fluoride ion and its implications on the epidemiological manifestations of the disease only in certain vulnerable segments of the community, while sparing others who are on more wholesome dietaries. Study of the physical chemistry of such interactions with tea and tamarind has given promising results. The results of physio-chemical studies which are of a probing nature were presented as B C Guha Memorial Lectures of the Indian Science Congress in Tirupati (1983).

#### **(4) Hepatic Pathology**

In the field of hepatic pathology, in addition to his studies of nutritional disorders of the liver in men and experimental animals, Sriramachari has been deeply involved in two other major studies. One of these relates to exhaustive and long-term work on Indian childhood cirrhosis; which was started in collaboration with late Prof. S T Achar and his successors Dr V Balagopal Raju and Dr N Sunderavalli. Thus, he is associated for over 30 years with one of the most exhaustive cross-sectional and long-term follow up studies of a clinico-pathological nature on this unique disease entity. The work was published initially in 1956<sup>16</sup> and has later been presented in several national forums. Multiple and serial liver biopsies have been performed over long periods of time on the same children with suitable treatment. This study, involving more than 1600 patients, has shed new light on the natural history of the disease.

This approach, based on dynamic pathology, is at variance with the prevailing view that the disease is associated with an invariable fatal termination. In a large proportion of cases, recovery and return to normalcy is possible under the influence of continued steroid therapy. This controversial subject, which has engaged the attention of several pathologists, is now converging towards some of the basic hypotheses put forward by the so-called 'Madras group' of which Sriramachari is an integral component. Apart from the impending publication of the report of the ICMR's multicentric study, Sriramachari and his colleagues plan to write a monograph on the subject.

Sriramachari has published an Illustrated Atlas on 'Histopathology of the liver', with particular reference to hepatitis and cirrhosis work done under the auspices of the ICMR.

Yet another major contribution in the field of hepatic pathology has come from his current work on the pathology of non-cirrhotic portal fibrosis, wherein he has shown that as opposed to the generally held view of 'obliterative' or 'occlusive' changes in the portal veins, there is actually a 'dilatation' of the portal veins at all stages of the disease. However, in view of radical differences among experts, his work has not yet been published apart from presentation at meetings of the Indian Association of Pathologists.

#### **(5) Medical Educational Technology**

As founder-Director of the Institute of Pathology (formerly known as the Indian Registry of Pathology) of the ICMR, Sriramachari had undertaken the challenging task of mass production and distribution of low-cost teaching aids in the field of pathology. For this purpose, he and



his colleagues developed an indigenous, self-reliant and appropriate technology of mass production of uniform high quality duplicates at phenomenally low cost of illustrations in colour and black and white of pathological specimens, X-rays, CT scans and above all photomicrographs of pathological lesions covering a wide range of diseases. This work entailed a comprehensive methodology related to microscopic optics, control of glare, Callier Q factor, control of contrast, etc. in slide duplication and photochemistry of different species, brands of colour films and adoption of relatively cheap cinematographic films for scientific photography. During the course of his work, Sriramachari and his colleagues have taken 7 patents in the name of Indian Council of Medical Research. Thousands of slides of good quality have been supplied to over 60 medical colleges and scientific investigators. The development of this unique facility, which continues to enjoy good reputation for the last 15 years among the scientific circles as well as non-medical is a significant national contribution.

### (6) Neuropathology

Sriramachari is among the earliest neuropathologists in the country, having been trained in the field in the world-famous Armed Forces Institute of Pathology, Washington, DC under Dr Webb Haymaker. Subsequently, he had an opportunity to visit some of the advanced centres in USA, UK and Europe.

He has made notable contributions in the study of brain tumours by conventional methods and application of hitherto unrecognized topo-optical properties of biofilaments of certain brain tumours, such as collagen fibres by

administering birefringence and dichroism to native glial filaments<sup>17</sup>.

Sriramachari and his colleagues, from their studies on experimental neurolathyrism in monkeys, demonstrated for the first time the direct damage of the anterior horn cells in the spinal cord of monkeys subjected to infrathecal administration of the active number of *Lathyrus sativus* seeds. This work<sup>18,19</sup> done in association with late Prof P S Sarma and Prof K S Mani, has great bearing on the understanding of neurological evidence of potentially hazardous foodstuff. The recent work of Spencer and others on cycad nut induced ALS like neuropathies is opening up a new frontier in neurobiology. Hence, the pioneering publications of Sriramachari and his colleagues are of considerable significance.

In central Indian cities like Nagpur, there was a perennial problem of sudden illness and death of children in summer months. This was attributed to a variety of causes, notably virus infections. However, Sriramachari and Dr Patoria studied in great detail the brains of 15 children who died and showed conclusively that there was no viral infection; it was rather a manifestation of acute deaths due to heat hyperpyrexia in unprotected urban children<sup>20-22</sup>.

With increasing vehicular accidents, the pathogenesis sequelae and reversibility of global or widespread cerebral oedema have assumed great importance. In spite of therapeutic advances, there is lack of understanding of the underlying mechanisms. Much of the confusion in the available literature is due to inappropriate experimental models for the study of experimental cerebral oedema available.

The limitations and choice of suitable experimental animals based on lack of sound scientific anatomical, circulatory and physiological considerations, employing mouse or rat as experimental models by local intense cold injury, etc., have led to a plethora of contradictory information on the nature of the material that seeps into the brain substance and which is likely to be reabsorbed. In association with late Prof. S N Pathak and Dr D K Balani, Sriramachari, carried out an extensive study employing more than 370 monkeys. They were subjected to different types of extradural cerebral compression. This study has brought to light that the oedema is essentially of a watery or saline type and if treated promptly by release of compression, hyperosmotic agents, cortico-steroids results in reversibility of the lesions. A series of papers on the subject have been published<sup>23-25</sup>.

### **(7) Pathological and Toxicological Aspects of Bhopal Gas Disaster**

During the last one year, Sriramachari has been actively associated with two major national disasters. He participated as an observer on behalf of the Government of India in connection with the autopsy studies of the Air India "Kanishka" crash. More importantly, he is deeply involved with the Bhopal gas disaster right from the middle of December 1984 to date. As one of the representatives of the Indian Council of Medical Research, he organized several teams related to the immediate autopsy studies as well as toxicological investigations pertaining to the subsequent ill-effects on the survivors.

He and his colleagues carried out detailed histopathological and

ultrastructural studies of the different organs in the gas affected victims in record time by 1 January 1985. Thereafter, he concentrated on the toxicological aspects of the investigation. He played a crucial role in establishing the importance of high urinary thiocyanate excretion, both as a diagnostic and therapeutic biomonitoring agent for assessing the efficacy of sodium thiosulphate therapy right from the initial stages in February 1985.

With the help of his colleagues, he produced a direct proof, contrary to the claims of the Union Carbide Corporation, that MIC crosses the alveolar-capillary barrier and gets tagged on to the end terminal amino groups of haemoglobin. The demonstration of carbamylation of haemoglobin as a major biochemical event in the Bhopal gas disaster is next in importance to the continued high excretion of urinary thiocyanate. It was also shown by him and his colleagues that such an interaction had resulted in compensatory mechanisms, such as elevated 2-3-DPG levels and abnormalities in the arterial and venous blood gas oxygen and carbon dioxide levels. He is one of the chief participants in the double-blind control studies on the efficacy of sodium thiosulphate and the subsequent investigations.

Sriramachari is Fellow, Indian National Science Academy (1975); National Academy of Medical Sciences (1962); Indian Academy of Sciences (1967); and Andhra Pradesh Academy of Medical Sciences (1976). He has been recipient of Shakuntala and Basanti Devi Amir Chand Awards of the Indian Council of Medical Research; J C Medal of Hari Om Trust of University Grants Commission; R V Rajan Oration of the National Academy of



Medical Sciences; B C Roy Award of the Medical Council of India and Dr Bishamber Nath Chopra Lecturership of the Indian National Science Academy. He has delivered a number of orations.

### Selected Publications

1. *Indian J med Res*, **8** (1954) 31.
2. *Indian J med Res*, **10** (1956) 944.
3. *Indian J Paed*, **20** (1952) 1.
4. *Lancet*, (ii) (1952) 661.
5. *Indian J Path*, **1** (1958) 105.
6. *Indian J med Sci*, **8** (1954) 433.
7. *Indian J med Res*, **46** (1958) 121.
8. *Indian J Path*, **2** (1959) 166.
9. *Indian J Path*, **2** (1959) 227.
10. *Indian J Path*, **1** (1958) 27.
11. *Indian J Path*, **1** (1958) 105.
12. *Indian J med Res*, **46** (1958) 121.
13. *Indian J med Res*, **50** (1962) 920.
14. *Indian J med Sci*, **11** (1957) 405.
15. *J clin Path*, **5** (1952) 346.
16. *J Paed*, **57** (1956) 744.
17. *Proc Int Congress of Neuropathology*, 1970 546-47.
18. *Nature, Lond*, (1967).
19. *Indian J med Res*, **59** (1971) 880.
20. *Indian J med Res, Tech Rep Series No 3*, Indian Council of Medical Research, New Delhi.
21. *Indian J med Res*, **64** (1976) 296.
22. *Proc Eleventh World Congress of Neurology*, 1977, 406.
23. *Indian J med Res*, **60** (1972) 859.
24. *Indian J med Res*, **61** (1973) 1550.
25. *In Use of non-human primates in biomedical research*, edited by M R N Prasad and T C Anand Kumar.

## P V Sukhatme

How much food does man need? Have we adapted ourselves to such low levels of intake and body weight that half of us are caught in a vicious circle of low intake leading to low productivity, low productivity leading to poverty and still lower intakes? The controversy started with my paper on the world's hunger, which I had the privilege to read at the joint meeting of the Royal Statistical Society and the Nutrition Society of Great Britain<sup>1</sup>. The finding was that the extent of undernutrition in the third world was not even one third as large as published by Lord Boyd Orr, the first Director General of FAO.

With the development of high yielding varieties of rice and wheat in the early sixties, the controversy shifted to the quality aspect of diet. A UN Subcommittee on Nutrition reported that protein malnutrition was widespread and that unless our diet was supplemented with high quality protein, malnutrition will continue to pose a big threat to economic development. To increase the intake of animal products was clearly difficult with the income levels which prevailed, but food technology could help to supplement diet with amino acids at a fraction of the additional cost and yet make the diet comparable in quality with what people ate in the west. However, even this approach became unnecessary. Analysis of diets

actually eaten showed that if a diet is adequate in calories, it is usually adequate in protein<sup>2-4</sup>. In this sense, normal intakes of even vegetarian diets in India are quite well balanced. Furthermore, available data on nitrogen output on fixed nitrogen intake in adult individual maintaining body weight showed that variation in daily nitrogen output was non-random and autoregressive in pattern. The current concept that as the intake decreases below the recommended intake for protein, the risk of protein deficiency increases had, therefore, no valid basis to support it. Briefly, protein gap turned out to be a myth<sup>5,6</sup>.

These findings reopened the question of the meaning of adequate food intake and its relationship with income. On current theory, individuals of similar age, sex, body weight and level of activity have similar energy requirements, but they are fixed in an individual. Measurements of habitual intake in healthy individuals accordingly provide the basis for calculating them. They are published as averages for each of the several age-sex activity groups and represent the requirements of the 'reference' individuals for the respective groups. They are interpreted to mean that if a person ate less than the prescribed norm, he is undernourished and if he ate more, he is overnourished. Paradoxically, however, Widdowson and other workers

---

Honorary Professor, Department of Biometry, MACS Research Institute, Maharashtra Association for the Cultivation of Science, Pune-411004; Residence : Sankhyadarshan, 64/12, Erandavana, Pune-411004.



have consistently observed for the last 40 years that in every group of 20 healthy active people, there are at least two, one of whom ate twice the amount eaten by the other and yet all were engaged in similar activity. Widdowson's explanation is that some individuals are more efficient machines than others. Clearly, in equating the observed habitual intake of an individual with the genotypic component in this way, she assumed that the variance of the phenotypic value in a given population  $V(P)$  is additive, given by  $V(G) + V(E)$ , implying that the two components  $G$  and  $E$  of  $P$  are independent in an individual and  $V(E)$  is negligible relative to  $V(P)$ . However, there was no way in which the validity of these assumptions could be examined until longitudinal data become available.

Critical reanalysis of the available longitudinal data for intake and expenditure in adults engaged in fixed tasks around 1970 by English physiologists showed that intra-individual variation is a wide source of variation, which cannot be made negligible by averaging intake and expenditure over a week or even longer periods. Further, it was noticed that intra-individual variation of mean daily intake was larger than that of mean daily expenditure, indicating that intake is in part behavioural in nature. As a result, the time lag between the two was found to be variable and energy balanced cumulated to a figure often so large that it became apparent that the body could not possibly tolerate it without gain or loss of weight unless it was served by some homeostatic mechanism which worked for the good of the whole body and enabled the individual to regulate his metabolism by speeding it up or slowing it down as necessary for maintenance of health and work output.

There is no violation of the first law of thermodynamics, which is concerned with the energy conservation of the total system: internal work does not figure explicitly in the law. There was a clear recognition in the evidence that the nutritional status of an individual is related to the process (pattern) of variation in energy balance over time and that consequently an individual could not be called as energy undernourished or protein malnourished unless his intake fell below the lower limit of the self-regulated range<sup>7,8</sup>. On the other hand, the process view gave no guidance as to how the hard edges of the self-regulated range should be defined if intake is averaged over several days to give an habitual value.

The breakthrough to resolve the dilemma came with the finding that the structure of variance of habitual intake (requirement) of an individual is non-random and can be expressed as a sum of two components, one of which is stable, independent of the number of days over which intake is averaged to obtain habitual intake and the other is a local environmental component, which decreased with the number of days<sup>9-15</sup>. This stable component expressed as a ratio of the total intra-individual variance is clearly the coefficient of heritability representing variability within oneself, which gave the individual the power to regulate his metabolism. Evidently, this power comes from interaction between genetic entities in man and a sustained perturbation in his environment and indicates that man enjoys freedom over a wide though limited range in choosing paths of development as he advances in time. On available evidence, this range is estimated at 80-120% of the prescribed norm and implies that the body simply

works more efficiently at the lower end of the range<sup>12</sup>.

A similar phenomenon of uneven growth rates from month to month is observed in well nourished children in USA. There is no evidence that such children are less healthy than those showing steady growth around the median. Indeed, it is the steady growth, especially at extremes, that is more likely to be the cause of concern in ensuring homeostasis. The inference that uneven growth rates occur because of inadequate energy or protein intake has no basis whatsoever. This behaviour is not prefigured in genes but occurs through interaction<sup>16</sup>. It is the omission of this covariance term (interaction) that has led Beaton, Scrimshaw and others to grossly overstate the physiological deterministic basis of an individual's intake and energy balance in influencing body weight under current nutrition theory<sup>16</sup>.

The above results have far-reaching implications for the measurement of undernutrition and poverty. The existence of homeostatic range implies that variation in the phenotypic values, such as work output or body weight cannot be fully explained by variation in intake or energy balance. Thus, it is not unusual to find that a fixed supplement of calories, say 300, does not produce the same change in body weight in all individuals. Indeed, it is a common experience that at similar intakes and similar levels of activity some gain in weight, while others do not. This is as it should be even on biological grounds, as confirmed by large amounts of DNA remaining untranscribed and untranslated in the development of an individual. When a trait is a quantitative trait with several genes contributing to its expression and is also under the influence of a variety of

environmental factors, such as food, drinks, morbidity, etc. plasticity in response is to be expected. It is this plasticity arising from the polygenic character of the genotype that has endowed man with a wide latitude of a homeostatic range for his intake and enabled him to indulge in food under conditions of affluence without being overweight and obese. Equally, the same plasticity has served well people in the third world in that it has enabled them to regulate energy balance without detriment to their health and reduction of work output over a wide range. It is not then that we have adapted ourselves to low intake anymore than people in the west have adapted themselves to high intake with all the risks associated with it. Undernutrition undoubtedly exists in the third world, but the scale is much smaller than the conventional views believe.

Even if intakes are adequate, malnutrition can still exist due to water-borne gastric and other diseases under unhygienic conditions where these adequate intakes cannot be metabolized.

These biological facts make it imperative to revise our perspective about the problems of malnutrition incidental to poverty. If intakes according to biological needs are denied for financial shortage, it has to be recognized as real poverty and our efforts have to be concentrated on rectification of this *real poverty*.

The above results have generated widespread interest and intensive debate at the same time. The cause of the latter is the misgiving which people have about reliability of data arising from errors of measuring energy expenditure. Further progress requires that we urgently build a human calorimeter which can record flow



of heat dissipated while the subject is engaged in a standardized programme of work. This project has just been approved by the Department of Science and Technology:

The real merit of the above result lies in enabling us to distinguish between basic needs and greeds of man. The fact that man uses his food more efficiently at the lower range of intake carries the message that man can now use education as an instrument to bring about transformation in his life style and of others like him by developing self-reliance and altruism at the same time. Indira Community Kitchen represents the largest community effort based on this principle. By organizing the work like the assembly line in a factory, the poor are not only enabled to earn their own livelihood but are also able to help the other poor to buy their food at half the market prices. The Kitchen works on the principle of no profit no loss with some 20,000 people benefiting every day in Pune<sup>7</sup>. The same principle is readily extended to villages. A simple device like a fence built around the school campus enables students to assume responsibility for keeping the campus clean of garbage, grow trees of economic importance, play and interact with one another besides working as informants on cases of morbidity if any in houses adjacent to their own. Likewise, a fence around an open well helps to ensure that soil can act as an effective filter in controlling the density of coliforms and other pathogens. Household and community latrines connected to the biogas plant is another step in the same direction. Served with tanks fed from dung where necessary we find that the supply of gas for cooking from a biogas plant is adequate to meet the needs of the villagers. All that is needed is motivation

and urge to avail of the health facilities. We have already made considerable progress in the construction of soak pit latrines and find that hygiene and sanitation are being rapidly built into the life style of the villagers to supply them with basic needs. What is needed is a still more educational effort around appropriate social action starting from Balwadies all the way upwards, so that man is enabled to interact with culture on one hand and mind on the other. To city-dwellers this approach may appear trivial, but where man cannot live as humans do over two-thirds of the country's rural area and yet extravagant resources are spent on feeding programmes as in ICDS project, the theory of development of man, his mind and intellect should surely be of the utmost importance both for the Planning Commission and for the education reform now under way<sup>15</sup>.

### Selected Publications

1. Sukhatme P V, The world's hunger and future needs in food supplies, *J R Stat Soc, Ser A*, **124** (1961) 462-525.
2. Sukhatme P V, Size and nature of the protein gap, *Nutr Rev*, **28** (1970) 223-26.
3. Sukhatme P V, The incidence of protein deficiency in India. *Indian J med Res*, **57** (1969) 2170-85.
4. Sukhatme P V, India and the protein problem, *J Ecol Fd Nutr*, **1** (1972) 267-78.
5. Sukhatme P V, The protein problem: Its size and nature, *J R Stat Soc, Ser A*, **137** (1974) 166-99.
6. Sukhatme P V & Margen S, Models for protein deficiency, *Am J clin Nutr*, **31** (1978) 1237-56.
7. Sukhatme P V, An experiment in community kitchen: Its philosophy and implications for the lives of the poor, *Soc & Sci*, **5** (1982) 56-68.
8. Sukhatme P V, On measurement of poverty, *Econ Polit Wkly*, **16** (32) (1981) 1319-24.
9. Sukhatme P V, The process view of nutrition and its implications for frontiers of research, *Proceedings of the Indian Statistical Institute's Golden Jubilee International Conference on*

- 'Frontiers of Research in Agriculture', Calcutta edited by SK Roy (Indian Statistical Institute, Calcutta), 1982, 35-74.
10. Sukhatme P V, Measurement of undernutrition, *Econ Polit Wkly*, **17** (50) (1982) 2000-16.
  11. Sukhatme P V, Poverty and malnutrition, in *Newer concepts in nutrition and their implications for policy* (Maharashtra Assn for Cultivation of Science) 1982, 11-63.
  12. Margen S & Sukhatme P V, Autoregulatory homeostatic nature of energy balance, *Am J clin Nutr*, **35** (1982) 355-65.
  13. Sukhatme P V & Narain P, The genetic significance of intra-individual variation in energy requirement, in *WG Cochran's Impact on statistics*, edited by P S R S Rao and J Sedranck (John Wiley and Sons, New York) 1983.
  14. Sukhatme P V, The process view of nutrition and its significance for adaptive regulation, *Proceedings XI International Congress of Anthropology and Ethnology*, Vancouver, 20-25 August 1983.
  15. Sukhatme P V, An approach to rural development: Theory and application, *Proc Indian natn Sci Acad*, **50 A** (1) (1984) 11-32.
  16. Sukhatme P V, The nature of energy requirement and its implications for measurement of undernutrition, *Invited Address at the 45th Session of ISI*, Amsterdam, August 1985.



## K Sundaram

Soon after receiving my post-graduate degree in obstetrics and gynaecology in 1953, I was selected as a research scholar to work in the upgraded Department of Obstetrics & Gynaecology, Women & Children Hospital, Madras, under late Dr R K K Thampan. My research work was mostly clinically oriented and related to the areas of my specialization. I was pleasantly surprised that one of the papers we published<sup>1</sup> elicited such a fine response that it was included in the *Year Book of Obstetrics & Gynaecology*, edited by J P Greenhill (Year Book Publishers, Chicago) 1957, 108. Our paper on the causation of toxemias of pregnancy provided direct evidence of increased levels of mineral corticoids, subsequently identified as aldosterone, as the main contributory factor for salt retention and consequent oedema and hypertension in this condition.

During the same period, I had been curious to examine as to why one observed variable response and control of disease in cases of carcinoma of cervix to intra-cavitary radium therapy. I observed that in some tumours contiguous sectors showed good and poor response to radiation, demonstrating differential radiosensitivity to cell killing and patients showing a mixture of good and poor response were prone to get a local recurrence and metastasis. Looking back,

it is now well known that tumours are not always well oxygenated and the anoxic cells are relatively radioresistant. Even today, the methods for coping with anoxic cells continue to be a major problem in radiotherapy and continued efforts are on to develop chemical radiosensitizers and hyperthermia internationally.

In late 1956, I joined the Atomic Energy Establishment, which was then located at the Tata Memorial Hospital. At that time, though exfoliative cytology was applied in clinical diagnosis of cancer in developed countries, especially in USA, it had not been exploited in our institutions. Dr Hannah Peters and myself developed a technique for the diagnosis of dysfunctional uterine bleeding which found wide application<sup>2</sup>.

One of the problems, which was of current interest then and continues to be so, was the biological effects of low doses of ionizing radiation in biological systems. Though it was known then that in experimental animals the male gonads could recover from temporary sterility after moderately high doses of X-rays or gamma-rays (of course, this recovery is associated with a genetic damage expressed in F<sub>1</sub> generation), the response of the female gonads had not been studied extensively. Unlike the male gonads which have a spermatogenic cycle, the female

---

Director, Bio-Medical Group, Bhabha Atomic Research Centre, Trombay, Bombay-400085.

gonads carry a predetermined number of oocytes at birth with no further replenishment. Our studies were perhaps the first to demonstrate that primary oocytes are extremely radiosensitive and an exposure of 25 R to a new born mouse can result in permanent sterility<sup>3</sup>. These observations carried with them a number of implications in radiation protection of man, generating a number of studies in mammals and primates. It is indeed reassuring that mouse ovaries are not a model for human ovaries in regard to radiation sensitivity.

During the sixties, with the peak of testing of nuclear weapons in the atmosphere, there was great concern about the levels of fallout and the biological consequences. <sup>90</sup>Sr, one of the fission products of nuclear weapons, which behaves in many ways metabolically like calcium, and is, therefore, a bone seeker, was shown to induce bone cancer. Our studies demonstrated that depending upon the radioactivity contained in the bone, it could induce leukaemias and/or bone cancers. This led us to a tentative postulate that cell systems with a high rate of turnover are not only highly sensitive to the killing effects, but are also highly prone to malignancies of that cell system<sup>4</sup>. This has been subsequently confirmed by workers in USSR.

Gradually, my research interests drifted into immunology and cancer. This was a period when major advances were taking place in the understanding of basic mechanisms in immunology.

The "Clonal selection theory" propounded by Prof. F M Brunet was accepted widely. The demonstration of bacterial immobilization by lymph node cells immunized against the specific

bacterial antigen by G J V Nossal and J Lederberg directed our attention to the possibility of cell surface carrying antibodies and thus modifying the charge density of the cell membrane. Prof. E J Ambrose, Chester Beatty Research Institute, came to our laboratory in late 1965 as a Visiting Professor, along with an instrument "a modified cylindrical cell for the microelectrophoresis of suspended particles", which he had developed along with J Ryder. This provided us the first opportunity to further investigate and reconfirm the observations of Nossal and Lederberg by an independent, simple and quantitative technique. The results of our work not only confirmed the "clonal theory" but also provided additional information on the kinetics of antibody producing cells<sup>5,6</sup>. In addition, we could recognize two sub-populations of lymphocytes which we had designated as Type I and Type II in the non-immunized lymph node cells and an additional Type III (antibody producing) cells in the immunized animal. Subsequent work resulted in identification of Type I cells as T cells and Type II cells as B cells, which are now well recognized classes of lymphocytes in immunology. These principles have been applied in the development of "cell-sorter", which has become an important instrument in biotechnology.

There has been a continuous debate with regard to the somatic mutation theory in carcinogenesis; the more recent discoveries of oncogenes are likely to provide further strength to the proponents of somatic mutation in the multistage process of carcinogenesis. The researches in the area of immunobiology of cancer and its control have had their ups and downs. Our current research efforts in



respect of the phenomenon of self and non-self recognition and methodologies to enhance non-self recognition specially induced by techniques of Allograft rejection appear to be promising in experimental animal tumours. We have generated adequate data in at least one tumour model to demonstrate that syngenic tumours will be rejected by its host if the host has been conditioned by a graft-vs-host reaction. This serves to indirectly demonstrate that the tumour has a changed antigenic profile, which is not adequate for the host to recognize it as "non-self". However, following a graft-vs-host reaction, the host is non-specifically sensitized to recognize fine differences between self and non-self antigens, leading to a rejection of the tumour. It would be interesting to follow up these leads in other animal tumour models and if this phenomenon can be generalized, then perhaps in the humans it would be possible to prevent recurrence of cancer.

As stated earlier, the biological effects of low doses of ionizing radiation on human population will continue to be a central point of concern for the continued acceptance and further progress in nuclear technology. One such opportunity for evaluating biological effects, if any, following life-time exposures of high natural background radiation, is provided by the population resident in the thorium-bearing beach sand areas of Kerala. A comprehensive demographic study on the radiation received by a representative sample involving over 12,000 individuals was carried out<sup>7</sup>. It will not be out of place to indicate here that this was the only comprehensive large scale study which provided a reasonable basis to assure that an average per capita annual dose of 500 mR is not likely to show an effect, which is

higher than in the case of those receiving a normal background radiation of 100 mR per year. Admittedly, one could argue that we had not excluded a genetic hazard. We would tend to agree; however, a population size of 70,000 is still not adequate to measure a doubling of spontaneous mutation rate, which is estimated to be around  $10^{-4}$  and  $10^{-5}$  per generation. It may be useful to mention that in studies on biochemical mutations in  $F_1$  offspring of the survivors of Hiroshima and Nagasaki concluded recently, there has been no evidence of increase in the mutation rate. Though theoretically one would expect an increase on the basis of linear dose-response relationship, for practical purposes one may conclude that at the levels of radiation exposure examined, the genetic harm, if any, would be subsumed by the variations in spontaneous mutations arising in such populations. My current research interests revolve around genotoxicity of chemicals in experimental model systems of whole animal bioassay and radiations in human populations.

### Selected Publications

1. Thampan R K K, Sundaram K, Ramaswamy S & Chamukuttan C K, Role of Adrenal Cortex in the causation of pregnancy, *J Obs Gyn India* (1956).
2. Sundaram K, Application of endometrial cytology in the diagnosis of uterine bleeding including a report on histochemical variations, *J Obs Gyn India*, **10** (1959) 2.
3. Sundaram K, The morphology, development and subsequent reproductive performance of the ovaries of Swiss mice given an X-ray dose at birth of 20r, *Proc Second International Conference of Radiation Research*, Harrogate, UK, August 1962.
4. Sundaram K, Some observations of late somatic effects of strontium-90 in rats, *Proc Symp on Cellular basis and etiology of late somatic effects*

- of ionizing radiations, edited by R J C Harris (Academic Press, London) 1963.
5. Sundaram K, Phondke G P & Ambrose E J, Electrophoretic mobilities of antigen-stimulated lymph node cells, *Immunology*, **12** (1967) 21.
6. Sundaram K, Phondke G P & Sundaresan P, *In vitro* studies on antibody production by lymph node cells using cell electrophoresis, *Immunology*, **13** (1967) 433.
7. Gopal-Ayengar A R, Sundaram K, Mistry K B, Sunta C M, Nambi K S V, Kathuria S P, Basu A S & David M, Evaluation of the long-term effects of high background radiation on selected population groups on the Kerala Coast, *Proc IAEA Symp on Peaceful Uses of Atomic Energy*, Vol. II (IAEA, Vienna) 1972, 31-51.
8. Phondke G P, Sundaresan P & Sundaram K, The extent of participation of electrostatic forces in antigen-antibody interactions, *Indian J exp Biol*, **8** (1970) 255.
9. Phondke G P, Sundaresan P & Sundaram K, Quantitative *in vitro* titration of antilymphocyte serum, *Indian J exp Biol*, **8** (1970) 329.
10. Phondke G P & Sundaram K, Cell electrophoretic studies on lymph node cells—Possible existence of two populations, *Immunology*, **21** (1971) 1.
11. Phondke G P, Gokhale Vidya K & Sundaram K, Fluorometric studies on the binding of antilymphocyte serum to lymph node cells, *Immunology*, **21** (1971) 437.
12. Sundaresan, P, Sundaram K & Phondke G P, Differential interaction of antilymphocyte serum with sub-populations of lymph node cells, *Immunology*, **23** (1972) 439.
13. Sundaram K & Chauhan P S, Dominant lethals in mutagenicity testing, *Lab Manual UNESCO/ICRO/DAE Training Course on Mutagenicity testing of drugs and chemicals*, BARC, Bombay, 1974, 1.
14. Phondke G P, Sundaresan P, Singh B & Sundaram K, Surface properties of T & B cells in leukaemic AKR mice, *Indian J Cancer*, **12** (1975) 405.
15. Chauhan P S & Sundaram K, Genetical toxicology: Scope for routine mutagenicity testing within the framework of classical toxicological practice, *Indian J Pharmacol*, **7** (1975) 104.
16. Chauhan Pawan S, Aravindakshan M, Aiyar A S & Sundaram K, Studies on dominant lethal mutations in third generation rats reared on an irradiated diet, *Int J Radiat Biol*, **38**(3) (1975) 215.
17. Aravindakshan M, Chauhan P S, Aiyar A S & Sundaram K, Studies on the possible mutagenicity of irradiated diets in rodents, *Proc Symp on Use of Radiation and Radioisotopes in Studies of Animal Production* (Dept of Atomic Energy, Bombay) 1975, 333.
18. George K P, Chaubey R C, Sundaram K & Gopal-Ayengar A R, Frequency of polyploid cells in the bone marrow of rats fed irradiated wheat, *Fd Cosmetic Toxicol*, **14** (1976) 289.
19. Ray P K, Thakur V S & Sundaram K, Anti-tumour immunity. II. Viability, tumorigenicity, and immunogenicity of neuraminidase treated tumour cells: Effective immunisation of animals with a tumour vaccine, *J natn Cancer Inst*, **56** (1976) 83.
20. Chaubey R C, Chauhan P S & Sundaram K, The evaluation of micronucleus test as an *in vivo* cytogenetic technique in mouse, *Mutat Res*, **53** (1978) 164.
21. Poduval T B, Seshadri M & Sundaram K, Lectin potentiation of BCG-contact-mediated antitumour action, *J natn Cancer Inst*, **65**(5) (1980) 909.
22. Chauhan P S, Aravindakshan M & Sundaram K, Genotypic sensitivity of seven inbred mouse strains to EMS induced dominant lethal mutations in mice, *J Am Coll Toxicol*, **3** (1984) 158.
23. Aravindakshan M, Chauhan P S & Sundaram K, Studies on germinal effects of Quercetin in mice, *Mutat Res*, **144** (1985) 99.



## M Swaminathan

I was initiated into research in organic chemistry in 1932 in the Annamalai University by the late Prof S N Chakravarti, himself a student of W H Perkin (Jr). He assigned to me the problem of synthesis of  $\psi$ -opianic acid, a product of hydrolysis of the alkaloid berberine. I achieved the synthesis in 1934. In 1935, I joined the Nutrition Research Laboratory, Coonoor (now National Institute of Nutrition, Hyderabad) and was assigned to a project on the chemical analysis of Indian foods. In 1937, soon after the discovery of nicotinic acid as the pellagra preventive vitamin by CA Elvehjem of USA, I developed a chemical method for the assay of nicotinic acid in foods and tissues and used it in studies on the role of nicotinic acid in nutrition. In 1940, soon after the isolation of pyridoxin (vitamin B<sub>6</sub>), I developed a chemical method for the assay of pyridoxin in foods. My appointment in 1942 as Assistant Professor of Biochemistry and Nutrition at the All India Institute of Hygiene and Public Health, Calcutta, gave me an opportunity to teach nutrition to students of DPH-course. I was appointed Reader in Biochemistry at Medical College, Jaipur, in 1947, and gained experience in teaching biochemistry to medical students.

I joined the Central Food Technological Research Institute, Mysore, in 1949 and continued my research activities for 27

years on various aspects of food science, technology and nutrition. The following were the important projects initiated and supervised by me:

(1) Development of low cost processed protein-rich foods based on blends of oilseed meals and weaning foods based on blends of cereals, oilseed meals and legumes and fortified with vitamins and minerals.

(2) Use of roots and tubers as partial substitutes for cereals.

(3) Development of grain substitutes based on blends of tuber flours and groundnut flour.

(4) Development of infant food based on buffalo milk.

(5) Studies on mutual and amino acid supplementation of proteins.

(6) Growth studies with preschool and school children to evaluate the supplementary value of low cost processed protein foods for poor Indian diets.

(7) Studies on the metabolism of proteins, calcium and phosphorus in children on poor Indian diets based on various cereals (with and without the addition of supplementary foods).

(8) Studies on the effect of insect infestation on the hygienic quality and nutritive value of cereals and pulses.

---

Formerly, Chairman, Applied Nutrition and Dietetics Department, Central Food Technological Research Institute, Mysore-13; Residence : Ganesh & Co Publishers, 41, Pondy Bazar, T Nagar, Madras-600017.

I attended the expert committee meeting of FAO/WHO on "Calcium requirements" in Rome in 1960, and the expert committee meeting on "Evaluation of protein quality" organized by the National Academy of Sciences, USA in Washington DC in 1962.

I am a founder editor of the *Indian Journal of Nutrition and Dietetics* and Associate Editor of the journal *Nutrition Reports International*, USA.

I was awarded along with three other colleagues (M R Chandrashekar, D S Bhatia and V Subrahmanyam), the Kidwai prize of ICAR in 1957 for our work on the development of infant food from buffalo milk. I was elected as an Honorary Member of the American Institute of Nutrition in 1969 for my contributions in the field of nutrition.

I have published over 200 research papers and over 30 review articles in collaboration with several coworkers. I have also written two books.

I record my indebtedness to late Dr S N Chakravarti who invited me to do research work under him. During my stay for 7 years at the Nutrition Research Laboratories, Coonoor (1935-42), I received encouragement and help from the Director, Dr W R Aykroyd and Asst Director, Dr R Passmore. I also wish to thank all my colleagues who worked with me at CFTRI, Mysore during 23 years of my stay there (1949-72). I record my special thanks to the late Dr V Subrahmanyam (Director from 1949 to 1961) and Dr H A Parpia (Director from 1961 to 1972) for their help and encouragement.

Among the scientists who collaborated with me in various projects are H B N

Murthy, M R Chandrashekar, G Rama Rao, M Narayana Rao, V Srinivasamurthy, Kantha Shurpalekar, S R Shurpalekar, S Kuppuswamy, K Krishnamurthy, P P Kurian, S Venkat Rao, V A Daniel, D Narayanaswamy, P K Tasker, M N Guttikar and Soma Kurian. I owe my success to their dedicated work.

### Selected Publications

1. Chakravarti S N & Swaminathan M, Synthesis of  $\psi$ -opianic acid, *J Indian chem Soc*, **11** (1934) 715.
2. Ranganathan S, Sundararajan A R & Swaminathan M, The chemical composition of 200 common foods, *Indian J med Res*, **24** (1937) 689.
3. Swaminathan M, A chemical method for the assay of nicotinic acid in biological materials, *Indian J med Res*, **26**(2) (1938) 427.
4. Swaminathan M, A chemical test for vitamin B<sub>6</sub> in foods, *Indian J med Res*, **28** (1940) 427.
5. Subrahmanyam V, Rama Rao G, Kuppuswamy S, Narayana Rao M & Swaminathan M, Development of Indian multipurpose food, *Food Sci*, **6** (1957) 76.
6. Chandrashekar M R, Bhatia D S, Swaminathan M & Subrahmanyam V, Production of infant food from buffalo milk, *Food Sci*, **6** (1951) 238.
7. Swaminathan M, Role of food technology in solving problems of nutrition in India, *Proceedings, VI International Congress on Nutrition, Livingstone, Edinburgh, 1964*, 149.
8. Swaminathan M, Availability of plant proteins, in *Newer methods of nutritional biochemistry*, Vol III (Academic Press, New York), 1967, 197.
9. Swaminathan M, The use of oilseeds and nuts in the feeding of infants and preschool children in the developing countries, *Pl Fd Human Nutr*, **1** (1969) 205.
10. Swaminathan M & Parpia H A B, The use of buffalo milk in the feeding of infants, *Wld Rev Nutr Dietet*, **8** (1968) 184.
11. Swaminathan M, The nutrition and feeding of infants and preschool children in the developing countries, *Wld Rev Nutr Dietet*, **9** (1968) 85.
12. Swaminathan M, Nutrition and the world food problem, *Bordens Rev Nutr Res*, **28**(1) (1967) 1-31.
13. Swaminathan M, *Essentials of food and nutrition*; Vol I. *Fundamental aspects*; Vol II. *Applied aspects* (Bangalore Printing and Publishing Co,



- Bangalore), 2nd Edn, 1965; Vol 1, pp 927; Vol 2, pp 654.
14. Swaminathan M, *Handbook of food and nutrition* (Bangalore Printing and Publishing Co, Bangalore), 4th Edn, 1984, pp 398.
  15. Swaminathan M, *Food science and experimental foods* (Bangalore Printing and Publishing Co, Bangalore), 1978, pp 482 (2nd Edn in press).
  16. Swaminathan M, *Biochemistry for medical students* (Geetha Book House, Mysore), 1979, pp 916.
  17. Swaminathan M, *Human nutrition and diet* (Bangalore Printing and Publishing Co, Bangalore), 1983, pp 160.
  18. Swaminathan M, *Principles of nutrition and dietetics* (Bangalore Printing and Publishing Co, Bangalore), 1986, pp 528.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

## G P Talwar

My research contributions have been mostly in three areas: mechanism of action of growth promoting and developmental hormones; immunology of leprosy; and immunology of reproduction.

### (1) Mechanism of Action of Hormones

#### (A) Estrogens

Estradiol has a marked uterotrophic action. We were among the first to put in evidence for the presence of highly specific binding proteins for estradiol-17 $\beta$  in organs which respond to estrogens<sup>1</sup>. The binding was non-covalent but of high affinity. Such proteins were not present in organs not affected by the hormone. The receptors were localized in both the cytoplasm and the nuclear compartments. The receptor protein did not bind cortisol and other steroid hormones. Discrimination was evident in binding of even the stereoisomer 17- $\alpha$ -estradiol, which is devoid of bioactivity.

An early effect of estrogens was the stimulation of DNA directed RNA synthesis. The importance of this event in the overall expression of hormonal effects was demonstrated through some simple but critical experiments. Local inhibition of RNA synthesis by topical application of actinomycin-D prevented the cornification of vaginal epithelium, while another organ such as the uterus in the same animal,

where RNA synthesis was not inhibited, responded normally to the administered hormone<sup>2</sup>. These early concepts have stood the test of time and form an essential part of our knowledge on the mechanism of action of this hormone. A limited role of cyclic AMP was also suggested by the enhanced uptake and incorporation of radioactive precursors in the uterus<sup>3</sup> and by the ability of cAMP to simulate some but not all effects of estradiol on the uterus<sup>4</sup>.

Activation of gene expression by estrogens and the mechanisms involved therein were the subject of study in male chicken which do not normally make egg yolk proteins but can be induced to do so under the influence of this female hormone. Administration of estrogens led to the expression of dormant genes. The hormone receptor complex was associated with chromatin. Initiation of transcription was an early effect of the hormone. Inhibition of DNA dependent RNA synthesis by actinomycin or treatment with compounds such as 8-aza-guanine blocked the induction of phosvitin<sup>5</sup>. Sequential exposure of the bird to estradiol shortened the latent period for the appearance of the egg yolk protein in circulation. The number of estradiol binding sites was also demonstrably increased<sup>6</sup>, the hormone inducing its own



receptors. An amplification of the capacity of the bird to produce the protein became evident from repeated exposure of the bird to the hormone<sup>7</sup>.

### (B) Pituitary Growth Hormone

The hormone stimulates somatic growth. It is important for bone and cartilage development in the early years of life. It is an anabolic hormone. It also influences carbohydrate and fat metabolism. More recent studies indicate the role of this hormone in the immune system and in particular in thymus metabolism. Our studies have provided unequivocal evidence for the presence of receptors for growth hormone on membranes of target cells, such as thymocytes<sup>8</sup>. Thymocytes respond *in vitro* to this hormone; an early effect is the increase in uptake of ions and metabolites. The uptake and incorporation of uridine into RNA is usable as a bioassay for the hormone, which is faster, and does not require hypophysectomized animals<sup>9</sup>. The hormone stimulates the metabolic activities of both the cortex and the medullary region of the thymus. Hypophysectomy or treatment with anti-growth hormone antibodies led to atrophy of the organ with reduced capacity for elicitation of immune response. These are recovered and enhanced by the administration of growth hormone. The hormone has thus a thymotropic role, a concept which our laboratory advanced based on many experimental evidences<sup>10</sup>. Using sophisticated approaches, it was demonstrated that about 65% of cells in thymus of young and growing rodents have receptors for this hormone. Their number diminishes with the age of the animal<sup>11</sup>. It was further shown that a possible reason for the lack of

responsiveness of cartilage of aged animal to growth hormone was the loss of receptors for the hormone on these cells in the course of development<sup>12</sup>. End organ responsiveness is an important feature in the action of this hormone, which continues to be secreted throughout life and is present in circulation. It affects only those organs which bear receptors for the hormone and not others. Some cells, such as peripheral blood lymphocytes in resting state, do not have receptors for this hormone but these are acquired when the cells are triggered to multiply by antigens or mitogens. The anabolic action of the hormone is exercised by enhanced synthesis of RNA and proteins<sup>13</sup>. The hormone activates the rate of synthesis of all classes of RNA in adult animals with little evidence of new species being induced<sup>14</sup>. With a larger number of protein synthesizing units and with the enhanced uptake of amino acids, cells make more proteins and the body has positive nitrogen balance<sup>15</sup>.

### (2) Immunology of Leprosy

Over 99% of people exposed to infection with *Mycobacterium leprae* do not develop the disease. Among the small number inflicted, the disease is manifest as a spectrum ranging from tuberculoid leprosy to lepromatous leprosy. In the former type, the lesion is circumscribed and the bacilli are few in number. On the other hand, in lepromatous leprosy (LL), the bacilli proliferate abundantly. *M. leprae* is an obligatory cellular parasite. A key question raised is on the mechanisms by which the host cell either restricts or promotes the proliferation of this bacterium. It was demonstrated that the leprosy patients do not have a generalized immune depression and are able to

respond normally to vaccination with typhoid<sup>16</sup>. They are, however, deficient in cell mediated immune functions to some *M. leprae* antigens<sup>17</sup>. Experimental methods were developed to study this deficit *in vitro*. Peripheral blood derived macrophages were cultivated and maintained in media enriched with serum for prolonged periods. The cells could be infected with *M. leprae*. A sensitive method was devised to determine the growth of *M. leprae* or lack of it in host cells. Advantage was taken of the absence in differentiated macrophages of thymidine kinase; thus a pulse of <sup>3</sup>H-thymidine labelled the multiplying mycobacteria with no radioactivity incorporated in the host cell nuclei<sup>18</sup>. This system enabled the delineation of the important role of lymphocytes in modulation of the macrophage activity; cells from tuberculoid patients inhibited the growth and those from LL patients had no restraining effect<sup>19</sup>. The action was exercised by products made by lymphocytes. Besides basic studies, this experimental system also found applications in screening of drugs as well as in determining within 3 weeks the drug resistance of mycobacteria in patients under treatment<sup>20</sup>. The time saving is substantial compared to 6-9 months required for getting the same answers in the mouse foot-pad system. The test has been rendered faster with recent improvements<sup>21</sup>.

Having determined that one of the deficits in lepromatous leprosy was the inability of the patient to mount a cell mediated immune (CMI) reaction against some specific *M. leprae* antigens, investigations were undertaken to develop approaches for breaking the tolerance to such antigens. One way to induce

responsiveness was the use of hapten modified *M. leprae*; acetoacetylated derivatives of *M. leprae* were shown to evoke responses associated with CMI functions from leucocytes of LL patients, which are otherwise anergic to *M. leprae*<sup>22</sup>. In another approach, a number of cultivable mycobacteria were tested as antigens with a panel of cells from tuberculoid leprosy (TT) patients. TT patients had evidence of infection, as also a capability for limiting the infection, an index of better immunity. Out of 16 mycobacteria tested, 5 were observed to produce reactions analogous to those of *M. leprae* with the reference lymphocytes. The rationale of the approach has been discussed elsewhere<sup>23</sup>. After a series of *in vitro* and *in vivo* tests, two mycobacteria, namely, *Mycobacterium w* (a hitherto unclassified nonpathogenic cultivable mycobacterium) and ICRC, were considered appropriate for the purpose. Both are under test as candidate vaccines. A single injection of autoclaved Mw converted to lepromin positivity status 62% of the LL and BL cases, who were persistently negative to lepromin<sup>24</sup>. The conversion was long lasting and was accompanied by evidence for the production of factors influencing macrophage function by leucocytes of immunized patients in challenge with *M. leprae*. This candidate vaccine has been cleared by the ICMR expert panels for Phase-II and Phase-III clinical trials.

Current research is seeking to develop simple methods for immunodiagnosis of leprosy. The aim of this study is eventually to identify the carriers of infection in the community. The incubation period of the disease is of several years, and during this period carriers in whom the bacilli proliferate are transmitters of infection to



others. Their detection and treatment with bactericidal drugs can limit the spread of the infection. An enzyme immunoassay measuring the IgM class of antibodies to a phenolic glycolipid unique to *M. leprae* provides a discriminatory test for diagnosis of multi-bacillary form of leprosy<sup>25</sup>. This test has now been converted to a dip-stick modality for use in laboratory and field conditions. The test is highly specific and negative in tuberculosis patients. Its limitation is in giving clear answers for treated LL patients as well as those belonging to paucibacillary forms of leprosy. Recent studies indicate that antigenic preparations from *Mycobacterium w* can extend the reliability of the test.

*M. leprae* does not grow in conventional media. Its supply from armadillo and nude mice is limited. Genomic libraries of *M. leprae* were prepared in pBR 322 and pSV GPT vectors. Some recombinants have been isolated which express in *E. coli* proteins serologically specific to *M. leprae*<sup>26</sup>. These could provide interesting antigens for immuno-diagnosis and other studies.

Debilities and deformities in leprosy arise from the peripheral nerve damage that the bacillus causes. Research is in progress to enquire on factors accounting for the predilection of this mycobacterium for the Schwann cells<sup>27</sup>.

### (3) Immunological Approaches to Control of Fertility

A new genre of vaccines were conceptualized and developed for regulation of fertility<sup>28</sup>. The birth control vaccines seek to induce antibodies intercepting a key hormone or a reproductive tract antigen critical for the

success of reproduction. The first vaccine proposed was against the human chorionic gonadotropin (hCG). hCG is an early signal of conception and is produced by the embryo before implantation. It is believed to provide the stimulus for sustenance of the functional activity of the corpus luteum and is thus important for establishment and maintenance of early pregnancy. Antibodies neutralizing hCG prevent the continuation of pregnancy in early phase. This concept has been verified by both active and passive immunization and efficacy confirmed in 4 subhuman primate species<sup>29,30</sup>.

hCG or sub-parts of it are *per se* not immunogenic in the human. They were made immunogenic by conjugation to tetanus toxoid<sup>31</sup>. The design of the vaccine was such so as to generate antibodies concomitantly against hCG and against tetanus<sup>32</sup>. The vaccine had thus an immunoprophylactic benefit against a health hazard besides the potential of fertility control. This vaccine has already undergone Phase-I clinical trials in six centres located in five countries. In 61 out of 63 women, antibodies were generated against hCG. The antibody response was reversible with titres declining to nearly zero levels in course of time. The duration in good responders was of 300-500 days. A booster injection of the vaccine increased the titres. Clearance studies showed that antibodies recognized and neutralized the bioactivity of hCG as tested *in vivo* and *in vitro*<sup>33</sup>. No significant side effects of immunization were noted, menstrual irregularity was retained with evidence of ovulation and normal libido, and metabolism and organ functions were normal<sup>34-36</sup>. The main limitation of this vaccine was the variability of titres and those with low titres were not protected

from pregnancy. In the course of subsequent research, two nontoxic adjuvants have been identified with ability to raise the antibody response by 2 to 3 folds. Another important advance is the enhancement of the intrinsic immunogenicity of the molecule. hCG is composed of two subunits, alpha and beta, and their association creates the conformation fitting best the target tissue receptors. Homologous alpha is not permissible owing to immunological cross-reaction with hFSH and hTSH.  $\beta$ -hCG, however, retains the ability to anneal with heterospecies alpha subunits and in fact the alpha-oLH- $\beta$ -hCG complex fits better the tissue receptors and has higher steroidogenic capacity than hCG. The immune response with this antigen was about 10 fold higher than with  $\beta$ -hCG-TT conjugates<sup>37</sup>. Another improved formulation devised is composed of a mixture of  $\beta$ -oLH and  $\beta$ -hCG each linked to two carriers, tetanus toxoid and cholera toxin chain B<sup>38</sup>. This vaccine is about 13 fold more immunogenic than the previous prototype. Both vaccines are going for clinical trials in several centres under the auspices of the Indian Council of Medical Research.

Most communicable diseases normally engender a variable degree of immune response in recipients. This is tolerated, as every one need not get infection. Birth control vaccines would, however, be used by fertile couples: hence the need to evolve an appropriate strategy for evoking response in most if not all recipients, in spite of different genetic backgrounds. This unusual problem of vaccinology is proposed to be resolved by the use of polyvalent vaccines consisting of more than one antigen of the reproductive tract linked to more than one carriers<sup>39</sup>.

Hyporesponse to one carrier may be compensated by better response to another. Such vaccines would in addition have the potential of multipoint interception together with diversification of the immunoprophylactic benefit.

#### (4) Immuno-diagnostics

We generated in 1980 the first hybrid cell clone in the country. The clone makes antibodies of high titre (10 million), high specificity and high affinity ( $K_a: 3 \times 10^{10}$  L/M) against hCG. It reacts with a conformational epitope common to  $\beta$ -subunit and in whole hormone. The clone has been acquired on lease by a leading US company for use in immuno-diagnostic kits. Sensitive and simple methods for detection of pregnancy and hCG synthesizing tumours have been developed<sup>40</sup>. Hybrid cell clones have also been generated against GnRH, progesterone, a number of sperm and egg antigens, *Salmonella typhus*, *E. histolytica*, *M. tuberculosis* and *M. leprae*. These clones help map the antigenic epitopes and are useful in cloning of genes of interest. Monoclonal antibodies from this source constitute key reagents in the array of diagnostic kits under development at NII.

#### Selected Publications

1. Talwar G P, Segal S J, Evans A & Davidson O W, The binding of estradiol in the uterus: A mechanism for derepression of RNA synthesis, *Proc natn Acad Sci (USA)*, **52** (1964) 1059-66.
2. Talwar G P & Segal S J, Prevention of hormone action by local application of actinomycin-D, *Proc natn Acad Sci (USA)*, **50** (1963) 226-30.
3. Sharma S K & Talwar G P, Action of cyclic adenosine 3',5'-monophosphate *in vitro* on the uptake and incorporation of uridine into ribonucleic acid in ovariectomized rat uterus, *J biol Chem*, **245** (1970) 1513-19.
4. Rao K N & Talwar G P, Action of oestradiol-17 $\beta$  and cyclic AMP on the synthesis of RNA



- phosphoproteins and phospholipids in the uterus of ovariectomized rats *in vitro*, *J Endocrinol*, **54** (1972) 215-26.
5. Talwar G P, Jaikhanani B L, Sopori M L, Venkatesan S, Grover A, Narayanan P R & Narasimhan C, Estradiol induced expression of genetic information: Synthesis of phosvitin in birds, in *Control of transcription*, edited by A Hollender and B B Biswas (Plenum Press, New York) 1973, 333-43.
  6. Sen K K, Gupta P D & Talwar G P, Intercellular localization of estrogens in chick liver: Amplification of the binding sites for the hormone on repeated exposure of the birds to the hormone. *J Steroid Biochem*, **6** (1975) 1223-27.
  7. Jaikhanani B L & Talwar G P, Estradiol induced synthesis of phosvitin amplified by successive exposure to hormone, *Nature, Lond (New Biol)*, **236** (1972) 239-40.
  8. Pandian M R, Gupta P D, Talwar G P & Avrameas S, Presence of the receptors for growth hormone on membranes of rat thymocytes, *Acta Endocrinol*, **78** (1975) 781-90.
  9. Saxena R K & Talwar G P, A new *in vitro* method for assay of the biological activity of growth hormone, *Acta Endocrinol*, **76** (1974) 248-59.
  10. Pandian M R & Talwar G P, Effect of growth hormone on the metabolism of thymus and on the immune response against erythrocytes, *J exp Med*, **134** (1971) 1095-1113.
  11. Hanjan S N S & Talwar G P, Effect of growth hormone on surface charges and electrophoretic mobility of thymocytes from young and old rats, *Mol Cell Endocrinol*, **3** (1975) 185-201.
  12. Talwar G P, Kumar N, Pandian M R & Gupta P D, Cellular and molecular correlates of the decline in responsiveness of cartilage and thymus to growth hormone in aged animals. *Mol Cell Endocrinol*, **1** (1974) 209-26.
  13. Talwar G P, Gupta S L & Gros F, Effect of growth hormone on RNA metabolism, Pt III. Nature and characteristics of nuclear subfraction stimulated by hormone treatment, *Biochem J*, **91** (1964) 565-72.
  14. Gupta S L & Talwar G P Effect of growth hormone on ribonucleic acid metabolism. The template activity of the chromatin and molecular species of RNA synthesized after treatment with the hormone, *Biochem J*, **110** (1968) 401-6.
  15. Talwar G P, Pandian M R, Kumar M, Hanjan S N S, Saxena R K, Krishnaraj R & Gupta S L, Mechanism of action of pituitary growth hormone, in *Progress of hormone research*, Vol 31 (Academic Press, New York) 1975, 141-74.
  16. Jha P, Balakrishnan K, Talwar G P & Bhutani L K, Status of humoral immunity in leprosy, *Int J Lepr*, **39** (1971) 14-19.
  17. Talwar G P, Krishnan A D, Mehra V L, Blum E A & Pearson J M H, Evaluation of cell mediated immune response in untreated cases of leprosy, *Clin exp Immunol*, **12** (1972) 195-203.
  18. Talwar G P, Krishnan A D & Gupta P D, Quantitative evaluation of the progress of intracellular infection *in vitro*: DNA synthesis in *Mycobacterium leprae* in cultivated blood monocytes, *Infect Immun*, **9**(1974) 187-91.
  19. Talwar G P, Krishnan A D, Jha P & Mehra V, Intracellular growth of an obligatory parasite *Mycobacterium leprae*—Host bacterial interactions, *Biochemie*, **56** (1974) 231-37.
  20. Krishnaprasad H, Nath I, Jaikhanani B L, Talwar G P, Ratan Singh & Seydel J K, Evaluation of known and potential antileprosy drugs using growth inhibition of *M. leprae* in cultivated human macrophages, in *Progress in immunology of leprosy* edited by G P Talwar *et al.* (Arnold Heinemann, New Delhi) 1983, 340-44.
  21. Chitamber S D, Band A H & Talwar G P, An *in vitro* test for assessment of intracellular viability and growth of mycobacteria, *Indian J med Res*, **82** (1985) 293-303.
  22. Fotedar A, Mustafa A S, Narang B S & Talwar G P, Improved leukocyte migration inhibition response of leukocyte from lepromatous leprosy patients with hapten modified *M. leprae*, *Clin exp Immunol*, **42** (1982) 317-24.
  23. Talwar G P *et al.*, Development of a potential anti-leprosy vaccine, *Leprosy in India*, **50** (A Special Issue) (1978) 488-599.
  24. Chaudhuri S, Fotedar A & Talwar G P, Lepromin conversion in repeatedly lepromin negative BL/LL patients after immunization with autoclaved *Mycobacterium W*, *Int J Lepr*, **51** (1983) 159-68.
  25. Ralhan R, Band A H, Roy A, Hajani G H, Sharma A K & Talwar G P, An enzyme immunoassay titrating IgM antibody against phenolic glycolipid puts in evidence active cases of lepromatous leprosy, *Indian J med Res*, **82** (1985) 110-15.
  26. Khandekar P, Munshi A, Sinha S, Gaur A & Talwar G P, Construction of genomic libraries of mycobacterial origin: Identification of

- recombinants encoding mycobacterial specific protein, *Int J Lepr* (1986) in press.
27. Band A H, Bhattacharya A & Talwar G P, Lack of *Mycobacterium leprae* specific uptake in Schwann cells, *Int J Lepr* (1986) in press.
28. Talwar G P, *Immunology of contraception* (Edward Arnold, London) 1980.
29. Tandon A, Das C, Jaikhani B L & Talwar G P, Efficacy of antibodies generated by Pr- $\beta$ -hCG-TT to terminate pregnancy in baboons: Its responsibility and rescue by medroxyprogesterone acetate, *Contraception*, **24** (1981) 83-95.
30. Talwar G P, Das C, Tandon A, Sharma M G, Salahuddin M & Dubey S K, Immunization against hCG efficacy and teratological studies in baboons, in *Non-human Primate Models for Study of Human Reproduction*, edited by T C Anand Kumar (S Karger, Basel) 1980, 190-201.
31. Talwar G P, Sharma N C, Dubey S K, Salahuddin M, Das C, Ramakrishnan S, Kumar S & Hingorani V, Isoimmunization against human chorionic gonadotropin with conjugates of processed  $\beta$ -subunit of hormone and tetanus toxoid, *Proc natn Acad Sci (USA)*, **73** (1976) 218-22.
32. Talwar G P, Structured vaccines for control of fertility and communicable diseases, in *Critical review in tropical medicine*, Vol 2, edited by R Chandra (Plenum Press, New York) 1984, 245-69.
33. Ramakrishnan S, Dubey S K, Das C, Salahuddin M, Talwar G P, Kumar S & Hingorani V, Influence of hCG and tetanus toxoid injection on the antibody titers in a subject immunized with Pr- $\beta$ -hCG-TT, *Contraception*, **13** (1976) 245-51.
34. Kumar S, Sharma N C, Bajaj J S, Talwar G P & Hingorani V, Clinical profile and toxicology studies on four women immunized with Pr- $\beta$ -hCG-TT, *Contraception*, **13** (1976) 253-68.
35. Nash H, Talwar G P, Segal S, Luukkainen T, Johansson E D B, Vasquez J, Coutinho E & Sundaram K, Observation on the antigenicity and clinical effects of a candidate antipregnancy vaccine:  $\beta$ -subunit of human chorionic gonadotropin linked to tetanus toxoid, *Fert Steril*, **34** (1980) 328-35.
36. Shahani S M, Kulkarni P P, Patel K L, Salahuddin M, Das C & Talwar G P, Clinical and immunological responses with Pr- $\beta$ -hCG-TT vaccine, *Contraception*, **25** (1982) 421-34.
37. Talwar G P, Om Singh, Sharma N C, Das C, Vinod Singh, Rao D N, Rao L V, Gaur A & Kaul S, An improved birth control vaccine inducing antibodies against human chorionic gonadotropin, *Proc natn Acad Sci (USA)* (1986) communicated.
38. Talwar G P, Om Singh, Vinod Singh, Rao D N, Sharma N C, Das C & Rao L V, Enhancement of anti-gonadotropin response to beta subunit of ovine luteinizing hormone by carrier conjugation and combination with beta subunit of human chorionic gonadotropin, *Fertil Steril*, (1986) in press.
39. Talwar G P, Immunological approaches to contraception, the need, basic premise and overview, in *Immunological approaches to contraception and promotion of fertility*, edited by G P Talwar (Plenum Press, New York) 1986, in press.
40. Talwar G P, Gaur A, Singh A K, Paul S & Gupta S K, Two simple and sensitive methods for detection of pregnancy and hCG synthesizing tumours amenable to both qualitative and quantitative assays; *Indian J med Res*, **77** (1983) 231-38.



## P N Tandon

In spite of the heavy responsibilities of developing safe patient care services in the demanding field of neurosurgery, over the past two decades Tandon's constant endeavour has been to pursue research, primarily clinical, often supported by a wide range of laboratory investigations. Many of the investigations have been of a multidisciplinary nature requiring close collaboration with colleagues from diverse basic and clinical disciplines, like neuroanatomy, neurophysiology, neuropathology, microbiology, nuclear medicine, neuroradiology, clinical neurology, neuro-otology, etc. The main thrust has been to study problems of national relevance, whose solutions must be found locally. The primary aim has been to reduce morbidity and mortality of neurological disorders prevalent in our society, while simultaneously contributing to the basic knowledge on such subjects. These studies have resulted in the publication of nearly 150 scientific papers, 5 monographs, chapters in a number of national and international books and a textbook of neurosurgery.

### **Delineation of Neurological/Neurosurgical Patterns in India**

Research in neurosciences, clinical or basic, was initiated very late in India, i.e. in the early 50's. There was little information about the incidence, pattern and natural

history of neurological diseases seen in our community. It was, therefore, only natural for most of us who introduced this discipline in the country to scientifically document the pattern of these maladies. Not unexpectedly, variations from the pattern seen and taught in the West where most of us received our speciality training were observed. Tandon's personal contributions in this field are mentioned below.

(i) A new syndrome, "Infantile tremor syndrome" occurring in India only, has been described. The clinical features, natural history, etiopathogenesis, pathology, as also electroencephalographic and radiological features have been delineated.

(ii) Anterior encephalocele, a developmental defect unusually common in India compared to the West has been studied. Its classification, diagnosis and treatment have been described. The utility of craniofacial osteotomy for total correction has been demonstrated and a new one-stage operation has been evolved.

(iii) Chronic persistent encephalitis as a cause of intractable epilepsy has been reported for the first time in India.

(iv) The etiology of subarachnoid haemorrhage has been studied. It has been demonstrated that contrary to the existing

belief, aneurysms are not uncommon as etiological factors in this condition.

(v) The etiology of paraplegia, its geographical variations and high incidence of transverse myelitis in our population, have been highlighted.

(vi) The variety and pattern of infective disorders as seen in India have been documented.

### **Studies on Tuberculosis of the Nervous System**

Tuberculosis of the nervous system continues to be a major problem in India. This is of little scientific interest to investigators in the advanced countries. It was, therefore, decided to take up this subject for in-depth studies. During the last 20 years, several facets of this problem at both clinical and laboratory levels have been studied. The work in this field has been recognized internationally, leading to invitations to write chapters on this subject for prestigious international books as also to deliver guest lectures at several centres abroad. These studies have been cited extensively in the world literature.

(i) The protean manifestations of the seen in our population have been delineated.

(ii) Its etiopathogenesis has been elucidated through experimental studies in guineapigs and monkeys. The role of immunity and sensitivity in the evolution of these lesions has been demonstrated.

(iii) The etiopathogenesis of hydrocephalus associated with tuberculous meningitis has been elucidated with the help of isotope studies of CSF pathways.

(iv) Unusual manifestations of spinal tuberculosis and their management have been described.

(v) Geographical variation in different parts of India in the incidence of tuberculoma of the brain has been demonstrated.

(vi) Atypical tubercle bacilli (scotochromogen) have been reported to be responsible for a case of brain tuberculoma. This is the only reported case in the world literature.

(vii) The CT lesion morphology of intracranial tuberculomas has been studied. This not only established the diagnostic criteria, now generally accepted, but has helped in developing the most appropriate therapeutic regime.

(viii) CAT scan features of tuberculous meningitis have been elucidated, resulting in the establishment of diagnostic and prognostic criteria.

(ix) Treatment of intracranial tuberculoma by medical therapy alone has been established. The work has been abstracted in the *Year Book of Neurology and Neurosurgery*.

### **Head Injury**

This has been a subject of continuing investigations over the last two and a half decades.

(i) Delineation of the pattern of head injury in the local community with a view to determining preventive measures and laying down guidelines for appropriate trauma service has been carried out.

(ii) Studies conducted on closed depressed fractures of the skull challenged the value of time honoured surgical treatment as a prophylactic against post-



traumatic epilepsy. This has since been independently verified by workers in the West.

(iii) A clinico-pathological study of cranio-cerebral erosion, unusually common in India, has been carried out and its management outlined.

(iv) A series of clinico-pathological and electrophysiological studies conducted on post-traumatic brainstem lesions have clarified several existing misconceptions regarding the incidence, clinical significance and prognosis of brainstem lesions in head injured patients. These studies have shown that contrary to the existing belief, a sizeable number of these patients can be saved through energetic management. This work has been abstracted in the *Year Book of Neurology and Neurosurgery*.

(v) An extensive study on temporal lobe lesions in head injury dealing with clinical features, diagnostic criteria, indications and techniques of surgery and prognosis challenged the existing views on the management of such lesions. Timely surgery has been shown to save life and reduce morbidity. This work has been abstracted in *Neurology Neurosurgery Abstracts*, and quoted in some important monographs on head injury.

### **Clinico-pathological Studies and Management of a Variety of Tumours**

(i) The incidence of various brain tumours as seen in India has been studied and compared with the information available from other centres in India and abroad.

(ii) Medulloblastomas have been studied to elucidate their morphogenesis, with special reference to differentiation.

(iii) Clinical, pathological, immunohistochemical and endocrinal studies of pituitary adenomas have challenged the existing classification of these tumours and provided new insight about their behaviour. A classification of haemorrhagic adenomas was proposed, which is now used by investigators in UK and USA.

(iv) Surgical management of dominant hemisphere gliomas has been evolved through careful study of a large number of cases. These investigations have demonstrated the superiority of our radical surgical approach to the commonly accepted practices for its management. This has been the subject of invited lectures at several centres abroad.

(v) Evidence has been collected to demonstrate the existence of primitive neuroectodermal tumours as an entity.

(vi) A number of papers have been published on some uncommon tumours, e.g. trigeminal neurofibromas, jugular foramen neurofibromas, intraventricular pituitary adenoma, aneurysmal bone cyst of spine, osteoblastoma of spine, and developmental tumours of the central nervous system.

### **Neuro-nuclear Medicine**

In collaboration with the Department of Nuclear Medicine, we were the first in the country to standardize and demonstrate the value of isotopic investigations in the diagnosis and management of a variety of lesions, utilizing RIHSA cisternography. CSF flow dynamics has been studied in a variety of lesions of the central nervous system, congenital, traumatic and inflammatory.

## Neuro-otology

In collaboration with the Department of Oto-rhino-laryngology, neuro-otology has been developed as a discipline. Investigations have been carried out on (i) the effect of raised intracranial pressure on auditory function, (ii) vestibular function in unconscious patients, and (iii) value of electronystagmography in diagnosis of posterior fossa lesions. Investigation (i) is the only detailed prospective study on this subject in the world.

## Clinical Electrophysiology

Electrographic study of sleep has been carried out in unconscious and brain damaged patients. Its utility in predicting the outcome of comatose patients has been demonstrated.

## Epilepsy

In collaboration with the Department of Neurology as a part of a multicentric national project, various facets of epilepsy, including epidemiological, psychological, social and therapeutic aspects have been studied. This has helped to determine the pattern of seizure disorders in India, the problems in their control, its psycho-social correlates and the results of treatment.

## Neurocysticercosis

Its diagnosis and treatment has been the subject of a detailed study. Some unsolved problems of etiopathogenesis have been outlined. CAT scan diagnostic criteria have been evolved in one of the largest series so far reported in the world literature.

## Plans for Future Studies

A multidisciplinary study on neural transplants has been initiated recently and it is planned to develop a sub-human

primate model for detailed morphological, neurophysiological and behavioural studies. This is the most exciting new field in neurobiology with immense potentialities for relief from a variety of neurological diseases.

A comparative study of the outcome of severely head injured patients treated as per the simple regime evolved by us and a high technology, costly regime practised at a premier centre in USA has demonstrated the usefulness of our regime, thus challenging some of the current management practices abroad. A further in-depth prospective study under Indo-US collaboration is continuing and another collaborative study with a centre in UK is planned.

## Selected Publications

1. Tandon P N, Singh B, Mohapatra L N, Kumar Mohan & Das Sarla, Experimental tuberculosis of the central nervous system, *Neurol India*, **8** (1970) 81-85.
2. Tandon P N & Pathak S N, *Tuberculosis of the central nervous system tropical neurology*, edited by J D Spillane (Oxford University Press, London) 1973, 37-62.
3. Tandon P N, Tuberculous meningitis (cranial & spinal), in *Handbook of clinical neurology*, Vol 33, edited by P J Vinken and G W Bruyn (Elsevier Publishing House, Amsterdam) 1978, Chap 12.
4. Jadhav W R, Sinha A, Tandon P N, Kacker S K & Banerji A K, Cold caloric test in altered states of consciousness, *Laryngoscope*, **81** (1971) 391.
5. Tandon P N, Brainstem haemorrhages in cranio-cerebral trauma, *Acta Neurol Scand*, **40** (1964) 375-85.
6. Bajpai P C, Tandon P N, Sharma N L & Misra P K, Infantile tremor syndrome, *Acta Neurol Scand*, **41** (1965) 473-86.
7. Tandon P N & Bajpai P C, The infantile tremor syndrome, in *Tropical neurology*, edited by J D Spillane (Oxford University Press) 1973, 14-19.
8. Tandon P N, Sinha A, Kacker S K, Saxena R K & Singh K, Auditory function in raised intracranial pressure, *J neurol Sci*, **16** (1973) 455-67.



9. Tandon P N, Meningoencephalocoele, *Acta Neurol Scand*, **46** (1976) 369.
10. Tandon P N, Singh B, Bhatia R & Banerji A K, Electroencephalographic study of sleep in cases of prolonged unconsciousness, *Neurol India*, **20** (Suppl II) (1972) 261-66.
11. Tandon P N, Rao M A P, Basu A K, Dar J & Das B S, 131-I-RIHSA CSF scanning in pediatric neurosurgical practice, *Neuro-Radiol*, **7** (1974) 119-23.
12. Dhawan I K & Tandon P N, Excision, repair and corrective surgery for fronto-ethmoidal meningoceles, *Child's Brain*, **9** (1982) 126-32.
13. Tandon P N, Cerebrospinal fluid circulation: Some unsolved problems, *Med Life Sci Engng*, **6** (1980) 25-36.
14. Tandon P N, Cerebral cysticercosis, *Neurosurg Rev*, **6** (1983) 119-28.
15. *Textbook of neurosurgery*, Vols 1 and 2, edited by B Ramamurthi and P N Tandon. (National Book Trust of India, New Delhi) 1980.

## M S Valiathan

Valiathan's interest in research was stimulated during his student days by the observation of patients with portal hypertension. His initial efforts centred on experimental studies on the role of liver in sodium retention in hepatic ascites and the presence of electrical activity in relation to biliary secretion. Interestingly, it was a casual suggestion from Prof. J B S Haldane which led him to investigate the secretory basis of the electrical activity of the liver. These early studies were carried out as experimental assignments during his general surgical training.

His professional evolution as a cardiac surgeon subsequently turned his interest to the burgeoning use of prosthetic materials in medical applications and more particularly in cardiovascular surgery. He soon came in contact with Dr Gott, who had made the serendipitous observation that heparin would ionically bond to a graphite-benzalkonium coated surface and enhance its antithrombogenicity. In a fruitful collaboration with Dr Gott at the Johns Hopkins Hospital, he carried out several studies to characterize the graphite-benzalkonium-heparin (GBH) surface and developed a new technique for resecting thoracic aortic aneurysms without systemic heparinization or external pump by using GBH coated shunts.

The complex problem of blood compatibility and the equally important challenge of developing cardiovascular devices at low cost in India found a ready response from Valiathan, who was instrumental in setting up a large interdisciplinary group at the Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum, for the development of biomaterials science and technology. Thanks to the integral links of the group with the modern hospital of the Institute, Valiathan and his colleagues successfully developed a tilting disc heart valve from titanium, sapphire and polyester cloth, a rigid shell oxygenator with integral heat exchanger and a crimped vascular graft from woven polyester, which are on the threshold of commercial production. These devices represent advanced technologies which owe as much to biomaterials science as to biomedical engineering and cardiovascular surgery.

More recently, the extensive experience in palliative open heart surgery has induced Valiathan to take an active interest in studying the aetiopathogenesis of endomyocardial fibrosis, which is an endemic disease of Kerala, Uganda, Brazil and some other parts of the world. This effort has mainly sought to link certain trace elements of the soil with the fibrotic



process of the endocardium. Regardless of whether the problem related to a basic study of endomyocardial fibrosis or the development of advanced cardiovascular technology, Valiathan's research interest was kindled and sustained primarily by clinical events.

In continuing to explore the mechanisms of endomyocardial fibrosis and the applications of biomaterials science, his endeavour will seek higher levels of absorption of science and technology in the practice of cardiovascular surgery.

### Selected Publications

1. Lalonde J B, Valiathan M S & Ballinger W F, Hepatic regulation of sodium and water in ascites, *J Am med Ass*, **187** (1964) 117.
2. Valiathan M S, Topaz S R & Ballinger W F, Electrical activity of the canine liver, *J surg Res*, **7** (1967) 186.
3. Valiathan M S, Whiffen J D, Weldon C S & Gott V L, Endocardial prosthetic junction thrombosis—An experimental study of etiological factors, *Trans Am Soc art int Organs*, **12** (1966) 174.
4. Topaz S R, Ameli M M, Morovati S S, Valiathan M S, Brown B G & Gott V L, Construction of a rigid case, double ventricle artificial heart, *Trans Am Soc art Organs*, **13** (1967) 294.
5. Gott V L, Whiffen J D & Valiathan M S, Graphite-benzalkonium-heparin coatings on plastics and metals, *Ann N Y Acad Sci*, **146** (1968) 21.
6. Valiathan M S, Weldon C S, Bender H W, Topaz S R & Gott V L, Resection of aneurysms of the descending thoracic aorta using a GBH coated shunt bypass, *J surg Res*, **8** (1968) 197.
7. Gott V L, Whiffen J D, Leininger R J, Falb R D & Valiathan M B, Thrombo-resistant surfaces, *Biblio Haemat*, **20** (1968) 863.
8. Valiathan M S, A new look at prosthetic materials, Hunterian lecture delivered at the Royal College of Surgeons, *Ann R Coll Surg*, **45** (1969) 131.
9. Valiathan M S & Shettigara U R, Biomedical materials, in *Manual of chemical technology*, Vol 3 (Ministry of Education, Government of India, New Delhi) 1977.
10. Valiathan M S & Bhuvaneshwar G S, Cardiac valvular prosthesis: An indigenous approach to development, *Indian Heart J*, **34** (1982) 387.
11. Venkatesan V S, Vijayan Lal A & Valiathan M S, A blood oxygenator from indigenous materials, functional evaluation using sheep lung as a deoxygenator, *Bull Mater Sci*, **5**(2). (1983) 97.
12. Bhuvaneshwar G S, Ramani A V & Valiathan M S, A tilting disc valve—Component materials and hydraulic function, *Bull Mater Sci*, **5**(2) (1983) 111.
13. Bhuvaneshwar G S, Valiathan M S, Lal A V & Kartha C C, Development of an arterial prosthesis: A preliminary report, *Indian J med Res*, **78** (1983) 556.

## N H Wadia

I started my career as an R M O to the National Hospital for Nervous Diseases, Maida Vale, and later as Registrar to Lord Brain at the London Hospital, Whitechapel, London. Among the papers contributed by me during the period of 30 years, four published in the journal "*Brain*" were considered as original observations. The paper "Some observations on atypical features in acoustic neuroma" was considered good enough to be summarized and commented upon in the *Medical Annual* (Yearbook 1957) as one of the two important neurology papers of the year.

### **Congenital Atlanto-Axial Dislocation**

Several papers were published on the clinical, radiological, pathological and management aspects of congenital atlanto-axial dislocations. In a paper presented to the Neurological Society of India in 1959, I pointed out that there was a rather high prevalence of craniovertebral anomaly, specially congenital atlanto-axial dislocation in India. Our subsequent papers attempted to clearly define the clinical, radiological and pathological features of the myelopathy complicating congenital atlanto-axial dislocation, distinguishing them from other craniovertebral anomalies and other types of spinal cord compression. This led to the

recognition of this entity all over India, leading to better management of patients with this disease.

### **Wilson's Disease**

Except for stray biochemically unproven reports of Wilson's disease, no literature existed in India till Wadia and Dastur's paper in 1963 on this subject. After laboratory facilities for diagnosis were established, considerable work regarding this disease was published from the J J Group of Hospitals, which stimulated work on it in other centres and now this disease is widely recognized in India. Of special research interest was our finding that a group of patients with Wilson's disease exhibited proximal lower limb weakness simulating myopathy. In fact, these patients had renal rickets presenting with myopathy and bone fracture. Attention was drawn to the proportionately high frequency of this mode of presentation in Wilson's disease at least in India. Today, we have on our records 49 patients from 36 families.

### **Nutritional Disorders of the Nervous System**

In a series of papers, we reported observations on several aspects of nutritional diseases due to malnutrition as seen in India, especially vitamin B<sub>12</sub>

---

Director of Neurology, Jaslok Hospital and Research Centre, Bombay and Consultant Neurologist, J J Group of Hospitals and Grant Medical College, Bombay-400036; Residence : A 8, B N Bhulabhai Desai Road, Bombay-400036.



neuromyelopathy from malabsorption, osteomalacic myopathy, and peripheral neuropathy with pellagra in nutritionally deprived urban alcoholics. A chapter in "*Progress in Clinical Medicine in India*", Series III (1979) summarized the work done by us and other workers in India.

### **Spinal Meningitis**

Substantial information on spinal meningitis and arachnoiditis was compiled especially pointing out the role of tuberculosis in this condition in tropical countries. It was stressed that early recognition and rapid administration of proper therapy lead to cure and avoidance of permanent disability.

### **Heredo-Familial Spinocerebellar Degeneration with Slow Eye Movements**

A series of papers dealing with the recognition of a new variety of heredo-familial spinocerebellar degeneration were published pointing out the common occurrence of this condition in India. While discussing its clinical features, postulations were made regarding the pathology of these slow saccades and their possible role in the physiology and understanding of fast eye movements. We also measured (accurately but with whatever crude instrumentation that was available in our department) the velocity and other parameters of these slow saccades. While discussing genetic and other observations, we drew attention to the involvement of the peripheral nervous system in this condition and autopsy confirmation of the clinical diagnosis. In a chapter contributed to the monograph "*Olivopontocerebellar Degeneration*", Duvoisin and Plaitakis (Raven Press, 1983), we summarized the total experiences with regard to this

disease. It was emphasized that it is an autosomal dominant disease and that proper genetic counselling should reduce the inheritable risk. In a recent postmortem monometric neuroanatomical study of one of our patients my colleagues and I have been able to confirm our original postulation regarding the location of the controlling neurones important for the execution of a saccade in the paramedian pontine reticular formation in the brainstem—an important anatomico-physiological observation culled through clinicopathological correlation.

### **Acute Haemorrhagic Conjunctivitis and Its Neurological Complications (Enterovirus 70 Disease)**

Several papers were published on this new virus disease which has affected millions all over the world over the decade 1970-1980. The main contribution was to recognize for the first time in the world its serious disabling neurological complications.

The clinical features, electromyography and complete serology of this disease have been worked out with the help of Indian and Japanese colleagues. Though like poliomyelitis it is incurable once a patient is affected, a timely warning and suggestions for prevention have been made. An editorial acknowledging these observations appeared in the *Lancet*, 1982.

### **SMON**

We have reported that this disease occurs not only in Japan, but in India too. Clioquinol, popularly marketed as Enterovioform and Mexaform, has been reported to have caused paralysis in 10,000 Japanese. By contrast, Indians who take large quantities of this drug are far

less affected. We have discussed the reasons for this difference on the basis of our clinical research on this problem.

### Multiple Sclerosis and HLA Antigens

We have reported that though multiple sclerosis is relatively rare in India compared to the west, yet it is more common among the small community of Parsees (Zoroastrians) than among other communities—religious groups living in India. We have discussed the related problems, which, in turn, may reflect on the larger problems of multiple sclerosis in the worldwide context.

### Selected Publications

1. Shepherd R H & Wadia N H, Some observations on atypical features in acoustic neuroma, *Brain*, **79** (1956) 282-318.
2. Wadia, Noshir & Williams Eirien, Bechets syndrome with neurological complications, *Brain*, **80** (1957) 59-71.
3. Wadia N H, Venous signs in cerebral angioma, *Brain*, **83** (1960) 425-31.
4. Wadia N H & Dastur D K, Wilson's disease in four Indian families—Clinical, genetical and biochemical aspects, *Neurology (Bombay)*, **11** (1963) 41-50.
5. Wadia N H, Manganese Intoxication in Indian miners, in *Tropical neurology*, edited by I Van Bogart, J Pareyra Kafer and G F Poch (Lopez Libereros Editors, Argentina) 1963, 271.
6. Wadia N H, Myelopathy complicating atlanto-axial dislocation (study of 28 cases), *Brain*, **90** (1967) 449-72.
7. Jeejeebhoy K N, Wadia N H & Desai H G, Role of vitamin B<sub>12</sub> deficiency in tropical 'nutritional' neuromyelopathy, *J Neurol Neurosurg Psychiat*, **30** (1967) 7-11.
8. Wadia N H & Dastur D K, Spinal meningitis with radiculomyelopathy—I. Clinical and radiological features, *J neurol Sci*, **8** (1969) 239-60.
9. Wadia N H & Swami R K, A New form of heredo-familial spinocerebellar degeneration with slow eye movements, *Brain*, **94** (1971) 359-74.
10. Wadia N H, Irani P F & Katrak S M, Lumbosacral radiculomyelopathy associated with pandemic acute haemorrhagic conjunctivitis, *Lancet* (i) (1973) 350-52.
11. Wadia N H, Nutrition deficiency disorders of the nervous system, in *Progress in clinical medicine in India*, Series III, edited by M M S Ahuja (Arnold-Heinemann Publishers, New Delhi) 1979, 487-510.
12. Wadia N H, Trikannad V S & Krishnaswamy P R, HLA antigens in multiple sclerosis amongst Indians, *J Neurol Neurosurg Psychiat*, **44** (1981) 849-51.
13. Wadia N H, Neurological disorders in India, in *Oxford textbook of medicine*, edited by D J Weatherall, J G G Ledingham and D A Warrell (Oxford Medical Publications, Oxford) 1983, 149-53.
14. Wadia N H, Wadia P N, Katrak S M & Misra V P, A study of the neurological disorders associated with acute haemorrhagic conjunctivitis, *J Neurol Neurosurg Psychiat*, **46** (1983) 599-610.
15. Wadia N H, A variety of olivopontocerebellar degeneration distinguished by slow eye movements and peripheral neuropathy, in *Olivopontocerebellar atrophies*, Vol. 41, edited by R C Duvoisin and A Plaitakis (Raven Press, New York) 1983, 144-77.



## P N Wahi

During his long research career, Wahi has been engaged in active research work in the field of cancer, in both experimental animals and human beings. The two commonest cancers in the country are cancer of the oral cavity in both males and females and cancer of the uterine cervix in females. These two cancers constitute about 50% of the total cancer morbidity and mortality figures. Wahi's indepth studies of these two cancers have yielded results which are being incorporated in the strategies for cancer control in India, making primary and secondary prevention of these cancers effective.

### Cancer of Cervix

This is the commonest cancer in the females in India, constituting as much as 80% cancers of the female genital tract. It is now known that this tumour does not arise *de novo* from the normal epithelium, but is preceded by certain epithelial abnormalities, detection of which could mean prevention of development of cancer. Also, cervical cancer, if detected at its preinvasive stage, is amenable to treatment with 100% survival rate. Wahi carried out extensive researches on experimental animals and human beings with a view to studying the changes in the uterine cervical mucosa, which progressively develops into cancer, to make early detection possible.

The animal studies were carried out on the mouse cervix after intravaginal painting with 3,4-benzpyrene. The progressive epithelial changes were studied by means of exfoliated cells in vaginal smears and histological examination of cervical epithelium. This led to the study of dysplasia, which lesion could be demonstrated prior to or co-existing with cancer. The findings showed that (1) dysplastic changes confined primarily to cytoplasm of the cells are more frequently reversible than when nuclei showed marked changes, (2) regression to normal epithelium occurred up to the stage of moderate dysplasia only, and (3) findings of marked dysplastic changes should always be looked upon with suspicion, as these invariably antedate cancer in experimental animals.

Differential and cytodifferential counts of the types of cells and cellular atypias respectively were taken and these seem to shed light on the understanding of cervical dysplastic lesions and their biological behaviour with regard to their progressive persistence or regression. Among the cytodifferential counts of the various cellular atypias, macronuclei hyperchromasia, macro and multiple nucleoli, number of squamoid cells and degree of chromatin condensation determine the progressive lesion.

Early epithelial changes were studied by phase contrast microscopy, and fluorescent microscopic examination of vaginal smears. These studies were undertaken to describe the cell structures in malignant lesions to find a method for early diagnosis of cervical cancer. Phase contrast microscopy was found to have an immediate direct use in the diagnosis of malignancies as well as in the less vital analysis of infectious and hormonal disturbances as manifested in gynaecological tract. It constituted a significant advance in the diagnosis of malignancy and also proved to be a valuable method for the study of living cells in the female genital tract. The cytoplasmic inclusions are very well defined; these cannot be seen by light microscopy.

Fluorescent microscopic studies also provided an important rapid and easy method of screening where smears can be screened under low power in a short time. In a solution of optimum acidity acridine orange and RNA combination fluorescence is due to varying degrees of polymerization of nucleic acid. Increase in DNA content of chromatin is found when cells show mitosis and polypoidy, characters which are common features of malignant cells. The technique has been found to be very superior, as no false positive cases have been recorded. It also does not require a highly skilled cytologist and is less time-consuming.

Wahi has been the pioneer in the country in studying revolutionary changes in the cervical epithelium during the development of malignancy by means of exfoliative cytology in both experimental animals and human beings. His studies on clinical, epidemiological and cytomorpho-

logical aspects of cervical dysplasias and their biological behaviour have been published in a monograph. These have revolutionized the understanding of etiopathogenesis of cervical cancer. Cervical dysplastic changes have been shown to be the forerunners of invasive cancer and detection of these changes would ensure cure or prevention of the tumour.

With the application of exfoliative cytology for the detection of dysplastic lesions, cancer of uterine cervix is now being considered as a preventable disease. The study has proved a great boon to Indian women, in whom this is the commonest cancer. In an over-populated country like India with a sizeable female population, mass screening will not be possible in the foreseeable future. But screening of high risk women is feasible. These are the women who were married early, had early sexual experiences, early pregnancy and multiple child births. Wahi carried out extensive field studies using cytomorphological and histomorphological techniques to define 'high risk women'. The screening programmes in this country are now confined to such women.

These multifaceted studies in the etiopathogenesis of the cancer of uterine cervix and its epidemiology have made a definite impact on the reduction of morbidity and rate of mortality due to this cancer in Indian women.

### **Study of Oral and Oropharyngeal Cancers in India**

These tumours constitute about 30.47% of all malignancies. An extensive epidemiological study was undertaken to establish the relationship or otherwise between tobacco use and oral cavity



cancer. This study which was carried out in the district of Mainpuri in UP revealed an incidence rate of 21.4 per 1,00,000 of the population—highest in the world. A significant correlation was observed between prevalence of oral cancer and the use of locally processed tobacco for chewing and/or smoking. This study was unique in that a direct measurement was made based on classification according to various environmental conditions of the population under study with the establishment of a systematic cancer registry at Mainpuri. The information collected was of great value—this was the first evidence obtained by strictly observing pathological requirements as well as using modern epidemiological methods for the measurement of the size of the problem in the community. A definite relationship between risk of developing oral cancer and tobacco chewing was established. It was found that the risk of developing oral cancer increased with increasing frequency of chewing and increased duration of keeping quid in the mouth. Differences in chewing and smoking habits determined the site of involvement.

Extensive studies of the two important lesions in the oral cavity which could be considered as precancerous—leukoplakia and submucous fibrosis—were carried out. Serum vitamin A studies showed vitamin A deficiency in cases of leukoplakia and oral carcinoma. This was established through spectrophotometric studies using destructive irradiation technique. Deficiency of vitamin A induces hyperkeratosis in oral mucosa, thus predisposing subsequent development of leukoplakia and cancer—an adjunct to the carcinogenic process.

As a result of clinical and histomorphological studies of submucous fibrosis, it was further observed that the severity and extent of the lesion and the habit (tobacco smoking and betel nut chewing) of the patient went together—clinical severity of the cases and the histopathological changes in the biopsy material. It was suggested that connective tissue changes may precede epithelial changes or may be concomitant. These changes seem to depend upon the combined effect of tobacco on the underlying connective tissue and the covering epithelium. However, the epithelial changes may be aggravated by abnormalities of the underlying connective tissue and blood vessels which act by interfering with metabolic changes or through direct effect of the products of degeneration or altered metabolism. The products of degeneration have been considered to have growth promoting properties and the epithelial changes may be secondary to the connective tissue changes. Another important conclusion was that the clinical severity and the extent of the lesion and atypical epithelial hyperplasia and the frequency and severity of hyalinization and fibrosis of juxta epithelial connective tissue went almost parallel. In tobacco non-users, orthokeratosis was seen predominantly in contrast to parakeratosis in tobacco users. High mitotic counts were found in the epithelium of tobacco users. In cases associated with cancer, parakeratosis was the prominent epithelial change.

With the recognition of submucous fibrosis as a precancerous lesion, further studies were undertaken on experimental animals with a chemical carcinogen to evaluate the epithelial changes—progressing from normal epithelium to the

development of submucous fibrosis and ultimately cancer. These included study of progressive cytomorphological, histomorphological, cytochemical and histochemical changes during carcinogenesis in hamster cheek pouch induced with 9,10-dimethyl-1,2-benzanthracene (DMBA). The significant cytochemical change observed was that as the cell changes from normal to neoplastic state through various dyskaryotic changes, the enzymatic activity increases progressively. It was suggested that the biological reactions involved in the differential counts of normal cells or growth of cancer cells are significantly associated with the presence of alkaline phosphatase. To further understand the biochemical changes in the cells undergoing carcinogenesis, electrophoretic studies of hamster cheek pouch tissue proteins during progressive experimental oral carcinogenesis induced with DMBA were carried out. This provided additional information on the subcellular metabolic fluxes which help in corroborating many of the routine and cytochemical findings. These provided a deeper insight into the metabolic fluxes related to anabolism and differentiation of cytostructures often involved in carcinogenesis.

A comparison between the mean values of individual fractions of normal cheek pouch with those found in the tissues at various stages of progressive carcinogenesis, i.e. submucous fibrosis, intraepithelial carcinoma and invasive carcinoma, was done. A significant variation was observed in fractions II, III and IV. Fraction II decreased, while fractions III and IV increased during progressive changes of DMBA-induced carcinogenesis. Slight decrease in fraction II in SMF and marked decrease in

intraepithelial carcinoma were correlated with the decrease in superficial type of cell which was observed in higher grades of dyskeryosis and in stages of intraepithelial carcinoma.

It was further shown by electrophoretic studies that in this experimental model protein changes in the hamster's serum during progressive stages of experimentally introduced carcinogenesis with DMBA consistently revealed the presence of five distinctly separated fractions corresponding to albumin, alpha I, alpha II, beta and gamma globulin of human serum. It was further observed that as the carcinogenic process progresses, the albumin fraction decreases. Alpha I and alpha II globulins showed a marked increase in SMF and cancer. The beta and gamma globulins were also found to increase in SMF and malignancy.

With the successful production of cancer in the mucous membrane of hamster cheek pouch by chemical carcinogenesis, and the study of progressive pathology from normal epithelium to malignancy, an experimental model was set up to study in detail the cytomorphological and histomorphological changes in the hamster cheek pouch treated with Mainpuri tobacco. Cytomorphological studies revealed dyskeryotic changes of superficial squamous cells. Histomorphological changes with submucous fibrosis and leukoplakia were observed in biopsy material from the cheek pouch. These cytological and histomorphological changes were found to be correlated with the human findings in oral leukoplakia and submucous fibrosis. The result of the study when compared with cytological and histologic findings of precancerous lesions encountered in human beings indicate that



tobacco is unmistakably responsible for the production of such lesions of the oral mucosa of the hamster and these closely simulate the lesions observed in human beings using Mainpuri tobacco over a variable period. Thus, for the first time a cause and effect relationship of tobacco and oral cancer was established. It was also proved beyond doubt that leukoplakia and submucous fibrosis are precancerous lesions, detection of which could prevent the development of cancer of the oral cavity in human beings.

To further examine the earliest lesions in the mucosal epithelium in the development of cancer in tobacco users, electron microscopic studies of the human oral mucosa after prolonged exposure to tobacco were undertaken. A notable feature in the tobacco treated mucosa was the development of a submicroscopic lesion at the epithelial connective tissue level. The abnormality was structurally similar to a condition termed microinvasion in other contexts. An interesting finding was that in the lesions produced by tobacco, this change disappeared with the cessation of exposure, whereas the extent of microinvasion during DMBA carcinogenesis increased even after exposure was discontinued. The important conclusion arrived at from these studies was that human precancerous lesions, i.e. leukoplakia and submucous fibrosis, are reversible if the person discontinues the use of tobacco. This has had a tremendous impact on the oral cancer control programme in the country.

The presence of microinvasion indicated a fundamental breakdown in epithelial-connective tissue interaction and long lasting disturbances of normal mucosal architecture. However, its prognostic

significance as an indicator of potential invasion on a large scale is yet to be proved.

These experimental and human studies have clearly established a close association between tobacco and oral cavity cancer and further recognized precancerous lesions, the detection of which could prevent the development of malignancy and thus help in reduction of oral cancer morbidity and mortality. The significance of these studies is realized when we remember that oral cancer accounts for as much as 47.0% of all malignancies in some parts of the country.

Realizing that early detection of oral cavity precancerous lesions and cancer has a tremendous potential in the prevention and cure of oral cancer, further studies were carried out to evaluate the role of exfoliative cytology in the detection of these lesions. Biopsy is not feasible as a screening procedure for the precancerous lesions in the population groups with high incidence of oral cancers (areas where betel, tobacco and *nass* are being used). Study of exfoliated mucosal cells (morphological and cytochemical) has proved valuable. Exfoliated cells from cases of submucous fibrosis present characteristic features which could be considered diagnostic. Alkaline phosphatase showed qualitative and semiquantitative increase in the cells (increased percentage score activity). As a result of this study, fairly characteristic changes in the exfoliated cells in oral lesions like SMF and leukoplakia were determined. This offers a distinct possibility of the use of oral smears in determining and investigating oral precancerous lesions. This was the first study of its kind in the world and the

results have been quoted in textbooks of cytology. Wahi was invited by the International Congress of Cytology in London to give an oration based on these findings.

Wahi's studies on cancers of the oral cavity and uterine cervix in experimental animals and human beings have yielded results which have helped in the identification of precancerous lesions and have made early detection of these lesions a distinct possibility. These are now being used in the implementation of cancer control programmes of the Government of India. Detection of these cancers at an early stage will considerably reduce the morbidity and mortality from cancer.

### Selected Publications

1. Wahi P N, Tandon H D & Bharadwaj T P, Adrenal cortex and hepatic cirrhosis. Role of adrenal cortex in evolution of carbon tetrachloride induced cirrhosis, *AMA Arch Path*, **62** (1956) 200-14.
2. Wahi P N, Tandon H D & Bharadwaj T P, Effect of cortisone on the progress of carbon tetrachloride induced cirrhosis, *AMA Arch Path*, **62** (1956) 215-17.
3. Wahi P N, Bodhke R R, Arora S & Srivastava M C, Serum vitamin A studies in leukoplakia and carcinoma of the oral cavity, *Indian J Path Bact*, **V** (1962) 10-16.
4. Wahi P N, Kehar Usha, Mali S & Misra G D, Histopathogenesis of carcinoma *in situ* of the uterine cervix, *Bull Wld Hlth Org*, **26** (1962) 661-74.
5. Wahi P N, Kehar Usha, Mali S & Bansal O P, Intra-epithelial carcinoma of the uterine cervix, *Acta Un Int Cont Cancer*, **XIX** (1963) 6-7.
6. Wahi P N, Kehar Usha & Lahiri B, Factors influencing oral and oropharyngeal cancers in India, *Br J Cancer*, **XIX** (1965) 642-60.
7. Wahi P N, Lahiri B, Kehar Usha & Arora S, Oral and oropharyngeal cancers in North India, *Br J Cancer*, **XIX** (1965) 627-41.
8. Wahi P N & Kehar Usha, Morphological and cytochemical studies of cells of precancerous lesions of the oral cavity, *Acta Cytol (Balt.)*, **10**(3) (1966) 173-78.
9. Wahi P N, Kapur V L, Luthra Usha K & Srivastava M P, Submucous fibrosis of the oral cavity—Clinical features, *Bull Wld Hlth Org*, **35** (1966) 789-92.
10. Wahi P N, Kapur V L, Luthra Usha K & Srivastava M P, Submucous fibrosis—Studies on epidemiology, *Bull Wld Hlth Org*, **35** (1966) 793-99.
11. Wahi P N, Luthra Usha K & Kapur V L, Submucous fibrosis of the oral cavity—Histomorphological studies, *Br J Cancer*, **XX** (1966) 676-87.
12. Wahi P N, Luthra Usha K & Kapur V L, Submucous fibrosis of the oral cavity—Morphological and cytochemical studies of the cells, *Indian J med Res*, **55** (1967) 374-79.
13. Kehar Usha & Wahi P N, Cytological and histological behaviour patterns of the premalignant lesions of the cervix in experimentally induced cervical dysplasias, *Acta Cytol*, **II**(1) (1967) 1-15.
14. Mali S, Wahi P N, Luthra Usha K & Kapur V L, Cancer of uterine cervix in Indian women, *Cancer*, **20**(5) (1967) 623-27.
15. Wahi P N, The epidemiology of oral and oropharyngeal cancer: A report of the study in Mainpuri District, Uttar Pradesh, India, *Bull Wld Hlth Org*, **38** (1968) 495-521.
16. Wahi P N, Mali S & Luthra Usha K, Factors influencing cancer of uterine cervix in North India, *Cancer*, **23**(5) (1969) 1221-26.
17. Bharadwaj V P, Atal P R, Luthra Usha K & Wahi P N, Electrophoretic studies in hamster serum proteins during progressive experimental oral carcinogenesis induced with DMBA, *Indian J med Res*, **57**(9) (1969) 1685-96.
18. Luthra Usha K, Bharadwaj V P & Wahi P N, Cytological and histological studies of the hamster cheek pouch treated with crude Mainpuri tobacco, *Indian J med Res*, **58**(5) (1970) 586-90.
19. Wahi P N, Cytology of oral precancerous and cancerous lesions, *Congress Oration, 4th In Cong of Cytology*, London, 1971.
20. Wahi P N, Epidemiology of oral carcinoma, *The Bishambar Nath Chopra Lecture, Proc natn Sc Acad*, **39B** (1973) 24-31.





**Sectional Committee—IX : Biochemistry and Biophysics**  
**(*Biochemistry, Biophysics, Molecular Biology,***  
***Microbiology and Immunology*)**





## P R Adiga

During the last 15 years, two major lines of research were undertaken in our laboratory: (i) discovery, isolation and properties of reproduction-specific carrier proteins for water-soluble vitamins, their hormonal induction and modulation, and (ii) biosynthesis of di- and polyamines, their metabolism during seedling development and hormonal regulation thereof in two representative higher plants, viz., *Lathyrus sativus* and *Cucumis sativus*.

### (1) Vitamin-carrier Proteins and Embryonic Development

In egg laying species, all essential nutrients are stored in the eggs in adequate quantities for use by the prospective embryos. In investigating the molecular basis of this enigmatic phenomenon, we found that in the domestic chicken (used as an oviparous model), egg deposition of riboflavin, thiamin and biotin is mediated by specific high affinity vitamin carrier proteins. These are not normal serum constituents (since they are absent in the male or immature animals of either sex), but are induced *de novo* either during egg-laying or upon artificial estrogenization in the maternal hepatocytes and oviduct with the sole objective of facilitating vitamin deposition in the yolk and white of the developing oocyte. These estrogen-inducible gene products were isolated in pure form, their

interaction with the respective vitamins investigated, specific radioimmunoassays for their individual quantification developed and the endocrine basis of their induction and modulation of their gene expression were defined<sup>1-5</sup>.

### Relevance to Mammalian Reproduction

It has been well established in clinical literature that during pregnancy in higher animals and humans, the rapidly developing conceptus actively concentrates the water-soluble vitamins from the maternal circulation against concentration gradients, despite the placental impermeability to the free vitamins or their coenzyme forms. However, the molecular basis of this physiological phenomenon was unknown. Based on our experience with the chicken model referred to above, we hypothesized that despite the marked difference in the pattern of embryonic development in the mammals *vis-à-vis* the oviparous species, the basic phenomenon of carrier-protein mediated vitamin delivery mechanisms might have been retained during evolutionary transition to ensure embryonic vitamin nutrition and survival and that the mammals may also utilize similar proteins as vitamin carriers for transplacental vitamin transport. To test this, the pregnant rat was chosen as a



mammalian model and the data obtained hitherto could be summarized as follows<sup>6-9</sup>: During pregnancy in the rat, there exist proteins in circulation which show immunological cross-reactivity with purified chicken egg riboflavin carrier protein (RCP) and thiamin carrier protein (TCP). Using specific radioimmunoassays for quantification, it could be clearly shown that (i) estradiol-17  $\beta$  specifically induces RCP and TCP in adult animals of either sex and the liver is the site of their synthesis and secretion into circulation. (ii) Circulatory levels of these vitamin carriers are modulated in concert with the changing estrogen levels during the estrous cycle and pregnancy. (iii) Selective passive immunoneutralization of pregnant rats using antisera to either homologous or heterologous carrier proteins during days 6-16 of gestation leads to profound fetal wastage, culminating in pregnancy termination. Detailed kinetic studies on [<sup>14</sup>C]-vitamin uptake by the conceptus in the presence and absence of the specific antisera reveal that blockage (> 95%) of vitamin efflux to the embryo on immunoneutralization is accompanied by cessation of its growth due to depletion of the vitamins and their coenzyme forms, thus culminating in fetal distress and hence pregnancy termination. The immunological evidence cited above for the occurrence of the above vitamin carriers in pregnant rats can be amply substantiated by actual purification and molecular characterization of these entities from the rat serum.

Availability of heterologous (chicken egg) carrier proteins prompted investigations on the feasibility of using the active immunization approach to suppress early pregnancy in the mammalian model. Active immunization of female rats of proven fertility with chicken vitamin

carriers and subsequent mating allowed embryonic implantation, but between days 6 and 9, there occurred 100% fetal resorption, as confirmed by a precipitous fall in maternal progesterone levels. The whole phenomenon was reversible in that successful pregnancy to term could be re-established if antibody titres were allowed to wane with time. Selective effect on fetal survival could be demonstrated, since actively immunized mothers were of normal health and their vitamin status and reproductive endocrine profiles remained unaffected.

It is remarkable that these reproduction-specific vitamin carriers are highly conserved throughout evolution, since similar proteins could be detected in pregnancy sera from a variety of higher mammals, such as sheep, cows, monkeys and humans. Not only is gross-immunological cross-reactivity retained, but even their gross molecular size and characteristics, affinities to selectively interact with the free vitamins, the site of synthesis and hormonal regulation of gene expression are scrupulously conserved. Recent studies with the sub-human primate model (*M. radiata*) not only confirmed the above basic phenomenon, but also revealed that immunoneutralization of the protein during gestation can have a grave consequence on fetal survival.

### Implications and Future Prospects

The following are among the important implications of these fundamental findings: (a) they explain the preferential accumulation of riboflavin, thiamin, etc., from the maternal supply line by the developing foetus in higher animals and primates; (b) they show for the first time that besides the vitamins, adequate

availability of their transport proteins ultimately determines the fetal vitamin status; (c) they suggest that genetic/endocrine disturbances in the production/functionality of these carrier proteins may be detrimental for fetal survival and proper progression of pregnancy, explaining some of the yet ill-defined cases of spontaneous/habitual abortion/fetal malformation; (d) they emphasize that continued estrogen secretion during pregnancy has an important physiological function in terms of fetal nutrition; (e) they raise the possibility that the approaches involving passive and/or active immunoneutralization of these reproduction-specific proteins offer a reversible and potential method of medical termination of pregnancy/menstrual regulation; (f) immunoneutralization of carrier proteins also offers for the first time an approach to precipitate selective fetal vitamin deficiency, thus opening new vistas of vitamin research in developmental biology. Finally, these fundamental findings illustrate handsomely how basic laboratory researches could have important implications in a variety of areas, such as maternal and child health, fertility control and family planning and in-born genetic abnormalities.

## **(2) Biosynthesis and Regulation of Polyamines in Higher Plants**

Another field of investigation to which substantial contributions were made is concerned with the biogenesis of polyamines, their metabolic and degradative pathways and environmental and hormonal regulation. The polyamines (PAs), spermine (SPR) and spermidine (SPD) and their diamine precursor putrescine (PUT) represent a set of evolutionally highly conserved small Mr ubiquitous organic polycations which play

vital roles as modulators of several life processes from enzyme activation and maintenance of ionic balance through regulation of growth and development to mediation of hormone action and progress of cell division. Recently, these amines have been implicated as playing important roles in normal and abnormal plant growth and development, pollination, sprouting of dormant buds, embryogenesis, photomorphogenesis and hormone-induced changes in plants. Our major contributions to the plant polyamine field (using *L. sativus* and *C. sativus* as representative models of higher plants) involved elucidation of arginine (ARG) → agmatine (AGM) → PUT → SPD → SPR pathway as the major sequence of events for polyamine elaboration during development. We could also demonstrate a parallel pathway, viz., homoarginine → homoagmatine → cadaverine → lysine in *L. sativus*. The latter incidentally gave a new biological role of homoarginine. The first and the rate-limiting enzyme of the above pathway, viz. arginine decarboxylase (ADC), was purified for the first time to homogeneity from *L. sativus* and its molecular and regulatory features were elucidated. A major breakthrough in these researches stemmed from the discovery that agmatine → PUT conversion in plants is not a simple two-step enzymic conversion with N-carbamyl putrescine (NCP) as intermediate and catalyzed by agmatine iminohydrolase (AIHase) and NCP amidohydrolase as postulated earlier, but is catalyzed by a versatile multifunctional protein, viz., putrescine synthase, with associated activities of AIHase, putrescine transcarbamylase (PTCase), ornithine transcarbamylase (OTCase) and carbamate kinase (CKase). An 'Agmatine cycle' was postulated to account for these results. This cycle of



reactions envisages the channelling of NCP generated *in situ* to the PTCase domain of the enzyme and coupling of PTCase activity to either OTCase or CKase in order to conserve the labile carbamyl phosphate otherwise dissipated. These reactions explained a number of hitherto inexplicable observations, including non-accumulation of NCP in the plant. These sets of reactions were later shown to be operative in PUT production, in *C. sativus* and other higher plants.

Among other major contributions made in the plant polyamine field are: (a) the discovery of an artifactual S-adenosyl L-methionine decarboxylase (SAMDC) activity (besides a prokaryotic type natural biosynthetic enzyme) in the plant due to hydrogen peroxide liberated as a consequence of diamine oxidase activity; (b) demonstration of an alternative pathway of spermidine synthesis involving carboxy SPD; (c) a novel pathway of homospermidine biosynthesis in *L. sativus*; (d) a versatile transamidinase catalyzing amine-amino acid conversion and homoarginine metabolism; (e) cytokinin modulation of ADC and growth in *C. sativus* cotyledon in culture; and (f) the phenomenon of uncoupling between polyamine biosynthesis and growth parameters in plants<sup>10-15</sup>.

### Future Prospects

Despite remarkable progress in elucidating the biosynthesis and regulatory role of polyamines in plants, admittedly several aspects of the metabolic machinery involved require clarification. Particularly relevant is the lack of knowledge regarding regulatory aspects of ADC as influenced by a variety of small molecules like  $K^+$  and osmolarity of the medium. The multiheaded putrescine synthase is an

attractive enzyme system to decipher the domain-directed flow of intermediates to various catalytic sites on a multifunctional protein. Finally, the molecular mechanism underlying plant growth regulation, embryogenesis and senescence by polyamines and hormonal influence thereon is a fruitful field for future investigation and is of both fundamental and applied interest.

### Selected Publications

1. Adiga P R & Murty C V R, Vitamin carrier proteins during embryonic development in birds and mammals, in *Ciba Foundation Symposium (No 98) in Molecular Biology of Egg Maturation* (Pitman, London) 1983, 111-36.
2. Murthy U S & Adiga P R, Estrogen induction of riboflavin binding protein in immature chicks: Kinetics and hormonal specificity, *Biochim biophys Acta*, **538** (1978) 364.
3. Muniyappa K & Adiga P R, Estrogen induced synthesis of thiamin-binding protein in immature chicks: Kinetics of induction, hormonal specificity and modulation, *Biochem J*, **186** (1980) 201.
4. Murty C V R & Adiga P R, Purification of biotin-binding protein from chicken egg yolk and comparison with avidin, *Biochim biophys Acta*, **786** (1984) 222.
5. Murty C V R & Adiga P R, Estrogen induction of biotin-binding protein in immature chicks: Kinetics, hormonal specificity and modulation, *Molec Cell Endocrin*, **40** (1985) 79-86.
6. Muniyappa K & Adiga P R, Occurrence and functional importance of riboflavin binding protein in the pregnant rat, *FEBS Lett*, **110** (1980) 207.
7. Murty C V R & Adiga P R, Pregnancy suppression by active immunization against riboflavin carrier protein, *Science, Wash*, **216** (1982) 191.
8. Adiga P R, Seshagiri P B, Malathy P V & Sandhya S V, *Proc International Meeting on Pregnancy Proteins in Animals*, edited by J Hau (Walter de Gruyter, Berlin/New York) 1986.
9. Krishnamurthy K, Surolia N & Adiga P R, Mechanism of fetal wastage following immunoneutralization of riboflavin carrier protein in the pregnant rat: Disturbance in flavin co-enzyme levels, *FEBS Lett*, **178** (1984) 87.

10. Adiga P R & Prasad G L, Biosynthesis and regulation of polyamines in higher plants, *J Pl Growth Regln*, **3** (1985) 203.
11. Ramakrishna S & Adiga P R, Arginine decarboxylase from *Lathyrus sativus* seedlings: Purification and properties, *Eur J Biochem*, **59** (1975) 377.
12. Srivenugopal K S & Adiga P R, Enzymic conversion of agmatine to putrescine in *Lathyrus sativus* seedlings: Purification and properties of a multifunctional enzyme (putrescine synthase), *J biol Chem*, **256** (1981) 9532.
13. Suresh M R & Adiga P R, Putrescine sensitive (artifactual) and insensitive (biosynthetic) S-adenosyl-L-methionine decarboxylase of *Lathyrus sativus* seedlings, *Eur J Biochem*, **79** (1977) 511.
14. Srivenugopal K S & Adiga P R, Co-existence of two pathways of spermidine biosynthesis in *Lathyrus sativus* seedlings, *FEBS Lett*, **112** (1980) 260.
15. Suresh M R, Ramakrishna S & Adiga P R, Relationship between arginine decarboxylase and putrescine levels in *Cucumis sativus* cotyledons, *Phytochemistry*, **17** (1978) 57.



## N Appaji Rao

The doctoral thesis work carried out by Appaji Rao in the Department of Biochemistry, Indian Institute of Science, Bangalore, pertained to the identification of the pathway for the biosynthesis and degradation of flavin coenzymes in plants. The enzymes involved in the synthesis of riboflavin 5'-phosphate (FMN), flavin adenine dinucleotide (FAD) as well as the enzymes degrading these coenzyme nucleotides were isolated to homogeneity and characterized. The post-doctoral work carried out at the University of Washington, Seattle, and at Scripps Clinic and Research Foundation, La Jolla, California, USA, led to an unequivocal identification of FMN as the flavin coenzyme of reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase of mitochondria and set at rest the controversy regarding the nature as well as role of this coenzyme in biological oxidations. The isolation of yeast mitochondria and identification of the electron transport chain opened up a new area of research.

The work at Bangalore carried out by him upon his return to India in 1964 has focused attention on three major areas, namely (1) Mechanisms of enzyme regulation and catalysis; (2) Biochemical mechanisms of drought resistance in plants; and (3) The relationship between

genetic disorders and inbreeding in the population of South India.

### (1) Mechanisms of Enzyme Regulation and Catalysis

(i) *Regulation by association-dissociation*: The early work resulted in the elucidation of the mechanism by which the activity of nucleotide pyrophosphatase was regulated by association-dissociation of the enzyme in the presence of adenosine 5'-phosphate (AMP). The enzyme was isolated as a dimer of mol wt 65,000 and exhibited an unusual biphasic time course of reaction. This was shown to be due to the conversion of the dimeric enzyme to a tetrameric form. The monomer isolated by the dissociation of the dimer by the addition of *p*-hydroxymercuribenzoate (pHMB) was no longer regulated by AMP. Desensitization could also be achieved without dissociation of the dimer by adsorbing and eluting the enzyme from Blue Sepharose. These results suggested that the AMP was a regulator of this enzyme involved in maintaining homeostasis of flavin and nicotinamide coenzyme nucleotides required for a number of oxidation-reduction reactions in the cell.

(ii) *Regulation by interaction at multiple sites*: Glutamine synthetase plays a vital role in the regulation of nitrogen

metabolism in plants. Using the homogeneous enzyme isolated in this laboratory from mung bean seedlings, it was established by careful and sophisticated kinetic analysis that the enzyme activity was coordinately regulated by interaction of the end products of the pathway at multiple sites on the enzyme. This type of regulation enables plants to channel the precursors among the different pathways of nitrogen metabolism. Glutamine synthetase from the fungus *Aspergillus niger* appears to play a crucial role in citric acid fermentation. Using kinetic methods, the role of metal ions in catalysis and production of citric acid by the organism was elucidated.

(iii) *Regulation of aspartate transcarbamylase by allosteric interaction, hysteresis and slow association-dissociation*: Another regulatory enzyme studied extensively in this laboratory is aspartate transcarbamylase (ATCase), which catalyzes the first unique reaction in pyrimidine nucleotide biosynthesis. This enzyme from *Escherichia coli* has served as a model protein to understand the mechanisms of allosteric interaction. The early studies of Appaji Rao's group involved the isolation of the enzyme, establishing the mechanism of the reaction catalyzed and the regulation of the enzyme activity by UMP, one of the end products of the pathway. Detailed kinetic as well as inactivation studies using specific reagents revealed that the catalytic centre was composed of histidine, arginine and tryptophan. The regulatory and catalytic sites were located near each other and not in two different subunits, as in the case of the *E. coli* enzyme. Mung bean ATCase exhibited multiple activity bands upon electrophoresis. Careful experiments showed that this was due to the

dissociation of the enzyme from a polymeric to a less organized state. This process was aided by the presence of the allosteric effectors, carbamyl phosphate and UMP. Superimposed upon this mode of regulation was the presence of hysteresis, which is defined as a slow response of a kinetic property of the enzyme to the binding of a ligand. Our studies with plant aspartate transcarbamylase revealed a new and novel phenomenon of regulation, viz., allosteric interaction linked to slow association-dissociation of the enzyme in the presence of effectors.

(iv) *Regulation of enzymes of folate metabolism by allosteric interactions and by activators*: Folic acid coenzymes supply the carbon fragments for the biosynthesis of metabolites, such as methionine, thymidine, purines, etc., which are essential for cell multiplication and growth. Our attention has been focussed on the first enzyme of the pathway in the interconversion of the folate pathway coenzymes, namely, serine hydroxymethyltransferase (SHMT).

This enzyme was isolated from several mammalian sources, such as monkey, sheep and human liver. Careful and detailed investigations established that the enzyme was indeed a regulatory protein with  $H_4$  folate and NADPH functioning as positive and NAD as negative allosteric effectors, while the enzyme present in the tissues of a healthy animal gave a sigmoid saturation pattern with  $H_4$  folate characteristic of the presence of homotropic interactions. The enzyme from tumour tissue gave a hyperbolic saturation pattern suggestive of the loss of regulatory constraints. However, purification of the enzyme from tumour tissue resulted in the regain of homotropic interactions with  $H_4$



folate, suggesting that some factor was probably lost during the purification step. Addition of an extract of fetal liver or neoplastic tissue devoid of the enzyme activity, to the purified normal or tumour enzymes, resulted in a hyperbolic saturation pattern. Preliminary results suggest that the factor is a protein. One possible biological significance of this observation is that the loss of homotropic interactions may result in a large increase in the enzyme activity at physiological concentrations of  $H_4$  folate. This could then meet the increased demand of thymidylate for the enhanced DNA synthesis characteristic of neoplastic tissues.

D-Cycloserine (DCS), an anti-tubercular drug, also inhibits tumour growth when the concentration of pyridoxal 5-phosphate (PALP) in the diet is low. Studies using circular dichroic, fluorescence and absorbance spectroscopy and activity measurement revealed that this mechanism-based inactivator was converting SHMT into the apoenzyme and Schiff's base of DCS with PALP was dissociated from the enzyme. This mechanism probably explains why the antineoplastic activity of this compound was related to the PALP status of the animal.

Another major interest in folate metabolism relates to its regulation in plants. As mentioned earlier, methionine is one of the end products of folate metabolism and this amino acid, essential for human nutrition, is limiting in several plants. It is hoped that an understanding of regulation of biosynthesis of methionine would provide reasons as to why this amino acid is limiting. Studies with dihydrofolate reductase (DHFR) and

SHMT have indicated that the properties of these enzymes from plant sources are different from those of enzymes present in bacteria and animals. A major difference is in the subunit nature of the plant DHFR, which is a multimeric protein made up of non-identical subunits, whereas animal and bacterial enzymes are monomeric. The plant SHMT is not a PALP enzyme, suggesting that its mechanism of reaction could be different from that of the animal enzyme.

## **(2) Biochemical Mechanisms of Drought Resistant Plants**

A major portion of agriculture in this country depends on the distribution as well as the intensity of the monsoon and in parts of the country which are not well irrigated crops are exposed to recurrent periods of drought. A common biochemical feature of many plants deprived of water is the accumulation of a compatible solute to maintain the turgidity very essential for crop growth as well as productivity. The concentration of free proline was increased 100-fold when ragi was grown under drought conditions. One reason for this dramatic increase was the complete inactivation of the enzymes that degrade proline, along with several-fold increase in the activity of enzymes synthesizing proline. A more dramatic change was the structural alteration in aspartate transcarbamylase, which resulted in the loss of regulatory constraints in the enzyme from stressed ragi plants. Rewatering the plants reversed these dramatic changes. Proline was a compatible solute, as indicated by its protective effect on key enzymes of proline metabolism; protein synthesis is reactivated in plants. These changes were not seen in plants which were not able to

withstand water stress. It can be concluded from our results that some plants have evolved mechanisms to withstand recurrent water deprivation during their growth and thus are better adapted to grow in semi-arid regions.

### (3) Genetic Disorders and Inbreeding in Human Populations

It is strongly believed that continued consanguinity, i.e., marriage between close relatives, leads to an increase in genetic disorders and decreased fertility and fecundity in human populations. A contrary view is also held that continued consanguinity in the South Indian population has led to the elimination of lethal genes from the breeding pool. A survey of the mentally retarded patients revealed that inborn errors of metabolism are present in this group, indicating that the mutant genes have not been eliminated from the population. Extrapolation of conclusions drawn using data from a highly selected group, like the mentally retarded, to the general population, is beset with several difficulties. Examination of more than 80,000 newborn children for the presence of amino acidopathies, interviewing the mothers for consanguinity and other parameters of fertility and fecundity has revealed that the incidence of the autosomal recessive disorders of amino acid metabolism in this population is approximately the same as in several outbred western populations. There was no significant change in fertility or fecundity between the consanguineous and non-consanguineous groups. These results are contrary to the widely held belief and demonstrate that genetic effects of inbreeding in large populations, such as those in South India, is minimal and should not be exaggerated. An examination of sick children clearly indicated correlation

between consanguinity and diseases caused by single gene defects. It can be concluded that although inbreeding may have no serious genetic consequence in large populations, it would have a marked effect on families with an affected child.

A common recurrent theme in all these studies is the fascination with the alteration in the activity of enzymes upon their interaction with small molecules or among their subunits or with the external environment. The intriguing consequences of such interactions have always provided the challenge to probe into the physiological significance of such observations.

### Selected Publications

1. Giri K V, Krishnaswamy P R & Appaji Rao N, On the occurrence of flavokinase activity in plants, *Nature, Lond*, **179** (1957) 1134-35.
2. Appaji Rao N, Cama H R, Kumar S A & Vaidyanathan C S, Alkaline beta-glycerophosphatase of green gram, *J biol Chem*, **235** (1960) 3353-56.
3. Huennekens F M, Felton S P, Appaji Rao N & Mackler B, Flavin component of DPNH dehydrogenase, *J biol Chem*, **236** (1961) 59.
4. Mackler B, Collip F J, Duncan H M, Appaji Rao N & Huennekens F M, An electron transport particle from yeast: Purification and properties, *J biol Chem*, **237** (1963) 2968-74.
5. Ravindranath S D & Appaji Rao N, Regulation of the metabolism of coenzyme nucleotides, *J scient ind Res*, **31** (1972) 210-19.
6. Appaji Rao N, Nishikimi M & Yagi K, Reactivity of D-amino acid oxidase with artificial electron acceptors, *Biochim biophys Acta*, **276** (1972) 350-63.
7. Achar B S, Savithri H S, Vaidyanathan C S & Appaji Rao N, Studies on plant aspartate transcarbamylase: Purification and properties of the enzyme from mung bean (*Phaseolus aureus*) seedlings, *Eur J Biochem*, **47** (1974) 15-22.
8. Ravindranath S D, Ashok Kumar A, Prema Kumar R, Vaidyanathan C S & Appaji Rao N, Mechanism of aromatic hydroxylations: Properties of a model for pyridine nucleotide



- dependent flavoprotein hydroxylases, *Archs Biochem Biophys*, **165** (1974) 478-84.
9. Harish Kumar P M, North J A, Mangum J H & Appaji Rao N, Cooperative interactions of tetrahydrofolates with purified pig kidney serine hydroxymethyl transferase and loss of this cooperativity in L1210 tumours and in tissues of mice bearing these tumours, *Proc natn Acad Sci, USA*, **73** (1976) 1950-53.
  10. Savithri H S, Vaidyanathan C S & Appaji Rao N, Plant aspartate transcarbamylase: Kinetic properties of the enzyme from mung bean (*Phaseolus aureus*) seedlings, *Proc Indian Acad Sci*, **87B** (1978) 67-79.
  11. Savithri H S, Vaidyanathan C S & Appaji Rao N, Plant aspartate transcarbamylase: Kinetic properties of the enzyme from mung bean (*Phaseolus aureus*) seedlings, *Proc Indian Acad Sci*, **87B** (1978) 81-94.
  12. Seethalakshmi S, Vaidyanathan C S & Appaji Rao N, Regulation of activity of mung bean (*Phaseolus aureus*) glutamine synthetase by amino acids and nucleotides, *Archs Biochem Biophys*, **196** (1979) 588-97.
  13. Narasimha Rao D & Appaji Rao N, Allosteric regulation of serine hydroxymethyltransferase from mung bean (*Phaseolus aureus*) seedlings, *Biochem biophys Res Commun*, **92** (1980) 1166-71.
  14. Reddy A R V, Balakrishnan C V, Sobhanaditya J, Ravindran S D, Ananthanarayana V S & Appaji Rao N, Comparison of the conformation and stability of the native dimeric, monomeric, tetrameric and the desensitized forms of the nucleotide pyrophosphatase from mung bean (*Phaseolus aureus*) seedlings, *J Biosci*, **2** (1980) 211-25.
  15. Sobhanaditya J & Appaji Rao N, Plant flavokinase: Affinity chromatographic procedure for the purification of the enzyme from mung bean (*Phaseolus aureus*) seeds and conformational changes on its interaction with orthophosphate, *Biochem J*, **197** (1981) 227-32.
  16. Appaji Rao N, Form and function of regulatory enzymes, Sreenivasaya Memorial Lecture, *J scient ind Res*, **41** (1982) 369-83.
  17. Rao D N & Appaji Rao N, Purification and regulatory properties of mung bean (*Vigna radiata*) serine hydroxymethyltransferase, *Pl Physiol*, **69** (1982) 11-18.
  18. Bittles A H, Radha Rama Devi A, Venkat Rao S & Appaji Rao N, A newborn screening programme for the detection of amino acid disorders in South India, *Biochem Rev*, **52** (1982) 20-24.
  19. Rao G S J, Prasad P V & Appaji Rao N, Hysteretic behaviour of mung bean (*Vigna radiata*) aspartate transcarbamylase, *Biochem Int*, **4** (1982) 503-8.
  20. Puneekar N S, Vaidyanathan C S & Appaji Rao N, Studies on *Aspergillus niger* glutamine synthetase: Regulation and enzyme levels by nitrogen sources and identification of active site residues, *J Biosci*, **6** (1984) 17-35.
  21. Kandpal Rajendra P & Appaji Rao N, Water stress induced alterations in ornithine aminotransferase of ragi (*Eleusine coracana*): Protection by proline against heat inactivation and denaturation by urea and guanidinium chloride, *J Biosci*, **6** (1984) 61-67.
  22. Prasad P V & Appaji Rao N, Interaction of rose bengal with mung bean aspartate transcarbamylase, *J Biosci*, **6** (1984) 613-24.
  23. Manohar R, Appu Rao A G & Appaji Rao N, Kinetic mechanism of the interaction of D-cycloserine with serine hydroxymethyltransferase, *Biochemistry*, **23** (1974) 4116-22.
  24. Kandpal Rajendra P & Appaji Rao N, Alterations in the biosynthesis of proteins and nucleic acids in finger millet (*Eleusine coracana*) seedlings during water stress and the effect of proline on protein biosynthesis, *Pl Sci*, **40** (1985) 73-79.
  25. Bittles A H, Radha Rama Devi A, Savithri H S, Sridhar Rajeswari & Appaji Rao N, Inbreeding and post-natal mortality in South India: Effects on the gene pool, *J Genet*, **64** (1985) 135-42.
  26. Varalakshmi K, Savithri H S & Appaji Rao N, Identification of amino acid residues essential for enzyme activity of sheep liver 5,10-methylenetetrahydrofolate reductase, *Biochem J*, (in press).

## B K Bachhawat

In the initial phase, studies on the metabolism of aminosugar in nervous tissues led to the elucidation of the pathway for the biosynthesis of aminosugars in the tissue. One of the new enzymes was also discovered, which degrades CMP-N acetyl neuraminic acid and this enzyme seems to have a regulatory role in the sialic acid metabolism. A study on the enzymatic activation of sulphate of developing brain led to the conclusion that there exist two distinct developmental parameters related to sulphation of glycolipids and glycosaminoglycans. It was for the first time that the pathway for the synthesis of cerebroside-3 sulphate by direct transfer of active sulphate to endogenous cerebroside was demonstrated.

Cerebroside-3 sulphate was known to be the major constituent which accounted for a genetically determined disease, metachromatic leucodystrophy. For the first time, it was shown that the lysosomal arylsulphatase A was deficient in this disease and this was the first demonstration that a lysosomal enzyme was involved in a sphingolipidosis. Arysulphatase A is now used as a marker for the diagnosis of this disease.

In systematic studies on the chemistry of glycosaminoglycans in nervous tissue, the presence of a large quantity of heparan

sulphate was demonstrated in this tissue for the first time. Subsequently, a number of in-born errors in metabolism leading to mental retardation were demonstrated in India for the first time. These diseases were related to the abnormal metabolism in glycoconjugates.

In a series of studies on lectins as a tool in glycoconjugate research, it was possible to develop methods for the purification of lysosomal enzymes with a lectin affinity column. A number of lysosomal enzymes were shown to be glycoprotein in nature. A new method for the isolation of a lectin from castor bean was developed using cross-linked guar gum. Using lectin affinity column, glycoprotein enzyme, invertase and antibody, an interesting method was developed for the synthesis of antibody enzyme monoconjugates. This led to a highly sensitive enzyme immunoassay for myoglobin.

A sialic acid binding lectin and a new hemagglutinin from horse-shoe crab were isolated and characterized.

During the work on the enzyme deficiency in in-born error in metabolism, it was felt that the liposome would be an ideal carrier of lysosomal enzyme to deliver it specifically to deficient cells. With this aim in view, studies on the interaction of a lectin and glycolipid liposome were



undertaken. The kinetic study revealed that this particular interaction was dependent on the density of glycoside residues, the chain length of the oligosaccharide fatty acids and cholesterol concentration.

Using this information, it was possible to develop a liposome model which was employed for the delivery of drug, enzymes and proteins. During this study, a new synthetic method for glycolipids was developed.

From this study, it was concluded that using different glycosides attached on the surface of liposome, one can specifically enrich liposome entrapped matter in the specific cells. For example,  $\beta$ -galactose liposomes entrapped material is preferentially taken up by the parenchymal cell, whereas mannoside liposome entrapped material enters into kupfer and sinusoidal cells of the liver. The endocytotic uptake was also found to be through respective glycoside receptors present on the surface of these cells. This work has in recent years been confirmed by various investigators in other parts of the world and it has been recognized as one of the important methods for targeting with liposomes.

When I look back into past achievements and contributions, I feel a certain degree of satisfaction that I had been able to develop a field of basic research and at the same time the results of these researches have been applied to clinical medicine.

I had been lucky to have during my entire research career a very intelligent, meritorious group of graduate students and colleagues and their intellectual and other inputs contributed to success in

these studies. Looking back in retrospect, I feel that a free and frank environment and a continued stimulative academic atmosphere which prevailed in my laboratory helped me and my students to achieve these objectives.

In a futuristic projection of this work, it may be mentioned that in recent years there had been increasing awareness on the role of glycoconjugates in the regulation of differentiation, growth and cell-cell interaction. It has also become clear from the work of my laboratory as well as elsewhere that the glycoconjugates, synthetic or natural, will play a major role in developing a new drug delivery system. Moreover, medicinal plants and other indigenous medicines will be looked into afresh, because in nature most of these drugs are in the form of a conjugate whether with glycosides or with peptides, etc. Its implication in oral administration and localized application will open up new vistas in the understanding of the mode of action of these drugs.

### Selected Publications

1. Austin J H, Balasubramaniam A S, Pattabiraman T N, Saraswati S, Basu D K & Bachhawat B K, Controlled study of enzyme activities in three human disorders of glycolipid metabolism (Gangosylism and metachromatic and globoid leukodystrophy), *J Neurochem*, **10**(11) (1963) 805-16.
2. Balasubramaniam A S & Bachhawat B K, Formation of cerebroside sulfate from 3'-phosphoadenosine-5'-phosphosulfate in sheep brain, *Biochem biophys Acta*, **106**(1) (1985) 218-20.
3. Shoyab Mohammed & Bachhawat B K, Enzyme degradation of cytidine 5'-monophosphate-N-acetylneuraminic acid, *Biochem J*, **102**(2) (1967) 13-14C.
4. Singh M & Bachhawat B K, Isolation and characterization of glycosaminoglycans in human brain of different groups, *J Neurochem*, **15**(3) (1968), 249-58.

5. Balasubramaniam A S & Bachhawat B K, Sulfate metabolism in brain, *Brain Res*, **20**(3) (1970) 341-60.
6. Podder S K, Surolia A & Bachhawat B K, On the specificity of carbohydrate-lectin recognition: The interaction of a lectin from *Ricinus communis* beans with simple saccharides and concanavalin A, *Eur J Biochem*, **44**(1) (1974) 151-60.
7. Balasubramaniam K A & Bachhawat B K, Purification, properties and glycoprotein nature of arylsulfatase A from sheep brain, *Biochem biophys Acta*, **403**(1) (1975) 113-21.
8. Surolia A, Bachhawat B K & Podder S K Interaction between lectin from *Ricinus communis* and liposomes containing gangliosides, *Nature, Lond*, **257** (5529) (1975) 802-4.
9. Pillai S & Bachhawat B K, A monoconjugate enzyme linked immunoassay, *FEBS Lett*, **90** (1978) 51-54.
10. Pillai S & Bachhawat B K, Affinity immobilization and negative cross-linking: A probe for tertiary and quaternary protein structure, *J molec Biol*, **131** (1979) 877-81.
11. Ghosh P, Das P K & Bachhawat B K, Selective uptake of liposomes by different cell types of liver through the involvement of liposomal surface glycosides, *Biochem Soc Trans*, **9**(6) (1981) 512-14.
12. Ghosh P, Das P K & Bachhawat B K, Targeting of liposomes towards cell types of rat liver through the involvement of liposomal surface glycosides, *Archs Biochem Biophys*, **213** (1982) 266-70.
13. Mohan Somasunderam, Dorai D T, Srimal S & Bachhawat B K, Binding studies of the sialic acid specific lectin from the horseshoe crab *Carcinoscorpius rotundicauda* with various sialo-glycoproteins, *Biochem J*, **203** (1982) 253-61.
14. Dorai D T, Mohan S, Srimal S & Bachhawat B K, On the multispecificity of carcinoscorpin—The sialic acid binding lectin from the horseshoe crab *Carcinoscorpius rotundicauda*. Recognition of glycerophosphate in membrane teichoic acids, *FEBS Lett*, **148** (1982) 98-102.



## John Barnabas

I embarked on my research career by accepting the challenging task of setting up a research unit at the Ahmednagar College, a small town college which was started in 1947 for the purpose of making higher education available to the people of interior Maharashtra. The long and rewarding association with the College began in 1954, when I initiated research activities in two small rooms of the Chemistry Department. My initial success in the application of paper chromatography as a versatile analytical tool<sup>1-3</sup>, the rewarding post-doctoral research experience at Yale University, USA (1958-59) and at the University of Groningen, the Netherlands (1960-61) and the recognition of the Chemistry Department of the College for post-graduate research by the University of Poona gave me the necessary impetus to start in 1963 a Biochemistry Department at Ahmednagar College. The department has grown in the course of years and has now attained the stature of a post-graduate school for biological studies, which not only trains students for MSc and PhD degrees in biochemistry and organic chemistry, but also serves as an interdisciplinary centre for research in life sciences.

In the early sixties, the field of biochemical evolution was in its infancy. I had, therefore, selected this field as my research theme. My earlier post-doctoral

work in the Netherlands on the structural characterization of a fusion hemoglobin called "Hemoglobin-Lepore Hollandia", which was published in *Nature*<sup>4</sup>, had greatly stimulated my interest in the hemoglobin molecule. I, therefore, used it as a model system for probing biochemical evolution.

In the first phase of my research at Ahmednagar, I had dealt with the diversity spectrum of hemoglobins in vertebrates. A prerequisite in such a study is the initial one of inventory taking, regardless of the level of diversity that one is dealing with. Through electrophoretic scans of hemoglobins of 53 animal species which covered 10 mammals, 29 birds, 6 reptiles, 1 amphibian and 7 teleost fishes, we established that multiple hemoglobins occur widely in vertebrates<sup>5,6</sup>. By characterizing through fingerprint maps a host of hemoglobins at the subunit level in the families *Bovidae* and *Muridae*, we demonstrated the presence of many electrophoretically silent hemoglobins in these groups; and more often than not, a common polypeptide chain was shown to be shared among within-species hemoglobins<sup>7-9</sup>. More significantly, the invariant two alpha-chain profile in hemoglobins, first observed by us in water-buffalo<sup>10</sup>, was shown to be a product of duplicated genes from a wider species coverage<sup>11</sup> as well as by their expression in

the fetal hemoglobins of this animal. Duplication at the hemoglobin loci is now known to be of widespread occurrence<sup>8,9</sup>.

In order to discern the evolutionary relationships among between-species hemoglobins, one requires in addition to the sequences of the component polypeptide chains, appropriate approaches for constructing phylogenetic trees based on these sequence data. Systematic sequence studies could not be undertaken in Ahmednagar College for want of facilities, although in the early 1960's we had made a painstaking effort to determine manually the amino acid composition of an easily crystallizable protein, beta-lactoglobulin, of bovids<sup>12</sup>. Through collaboration with Prof. Genji Matsuda of Nagasaki University, Japan, we contributed to the determination of the sequences of alpha and beta globins of langur, *Presbytis entellus*<sup>13</sup>.

My visit to Wayne State University, USA in 1969 to work with Prof. Morris Goodman made it possible for us to construct phylogenetic trees of globin sequences and in turn to establish that hemoglobin-myoglobin gene duplication probably took place in the primitive vertebrates after the divergence of lampreys, alpha-beta gene duplication in the primitive fishes, the beta-gamma duplication in the basal therians and beta-delta duplication in the higher primate stem<sup>14,15</sup>. More significantly, we developed mathematically proven methods for the construction of phylogenetic trees based on the maximum parsimony approach<sup>16,17</sup> as well as on the "additive hypothesis" approach<sup>18</sup>.

In Ahmednagar, we have developed a simple parsimony method<sup>19,20</sup>, which we call the "zip method" for the construction

of maximum parsimony amino acid sequences at the interior points of a given network. This method is equally applicable to nucleotide sequence data sets. A statement of the method and its working approach follows.

Given the amino acids at each of the exterior points (EPs)  $E_i$ ,  $i = 1, 2, \dots, n$  of a network, to find the amino acids at each interior point (IP)  $I_i$ ,  $i' = 1, 2, \dots, (n-2)$  such that the total network length  $L(N) = \sum m_r$  is minimum, where  $m_r$ , the length of the  $r$ th link, is determined by the number of base changes at the aligned positions of the codons present at opposite ends of the link.

Let  $E_{ij}$ ,  $i = 1, 2, \dots, n$  and  $j = 1, 2, \dots$  denote the  $j$ th codon at  $E_i$ . Zipping operation identifies amino acids at IPs that have the potential to minimize  $L(N)$ , whereas unzipping further establishes a final set of amino acids at IPs and EPs such that  $L(N)$  is minimum. The minimum  $L(N)$  is referred to as the parsimony length for that network.

In order to apply zipping operation, we dissect the set of IPs into subsets,  $C_1$  and  $C_2$ .

Case (i): For each  $I_i \in C_1$ , we consider three corresponding EPs. A point of  $C_1$  is characterized by two EPs as the first nearest neighbours (FNNs) and a unique EP and one IP as the second nearest neighbours (SNNs). From these three EPs, the one having the maximum number of codons is initially chosen as a select exterior point, say  $E_s$ . For  $j$ th codon  $E_{sj}$  at  $E_s$ , we form the set/s  $S_j$  of contributory codons.  $S_j$  has exactly three elements; (a)  $E_{sj} \in S_j$ , and (b) the other two elements are from respective FNN(s)/SNN such that the distance  $d(E_{sj}, E_{sj'})$  is the minimum



possible, where prime denotes the other two EPs. There can be more than one set of  $S_{j's}$  corresponding to a given  $E_{sj}$ . More sets of  $S_{j's}$  can be formed by taking the other two EPs as  $E_s$ . Using the elements of  $S_j$ ,  $j = 1, 2, \dots$ , we form a set  $T_j$  of codons with the following property:  $k$ th nucleotide  $t_{jpk}$  of  $p$ th codon  $t_{jp} \in T_j$  is the most common  $k$ th nucleotide of contributory codons of  $S_j$ . If such a common  $k$ th nucleotide does not exist, then  $t_{jpk}$  assumes all values of the  $k$ th nucleotide in contributory codons. The set  $V_r = U_j T_j$  is assigned at the interior point  $I_r$ .

Case (ii): For an IP in  $C_2$ , we consider only its two EPs (FNNs). For a point in  $C_2$ , there are two EPs as FNNs and two IPs (or two EPs when  $n = 4$ ) as SNNs. The procedure described for case (i) is applied to these two EPs to get  $V_r$ .

Now to assign codons to interior points, we apply either case (i) or case (ii) whichever is appropriate. We continue the operation in a stepwise manner till we reach the last interior point ( $I_{n-2}$ ).  $I_{n-2}$  will require the application of case (i).

For unzipping, we first assign superscripts to codons of  $I_r$ , the values of which are  $\Sigma mr$ 's. Since the value of a superscript of a codon at  $I_{n-2}$  represents  $L(N)$ , the codon with the minimum superscript is retained at  $I_{n-2}$  without superscript. Unzipping which starts at  $I_{n-2}$  selects link codons at each interior point and ends at  $I_1$ .

Using this method, mammalian globin gene phylogenies for different sets of myoglobins, alpha globins and beta globins were derived in the usual manner. From these phylogenies, evolutionary genetics of mammalian globins as well as basic patterns of mammalian evolution during Cenozoic history were discerned<sup>19</sup>.

Mammalian phylogeny derived from these data sets closely parallels that from traditional taxonomy.

An analysis of functional innovations in the hemoglobin molecule as it evolved from a monomer having high oxygen affinity and low cooperativity to a tetramer having low oxygen affinity and high cooperativity was carried out<sup>21</sup>. This analysis combines information from the ancestral amino acid sequences constructed at the branch points of the phylogenetic tree based on representative globin sequences with the information from the oxygenation curves of hemoglobins, their three-dimensional structure and the structural organization of globin genes. The role of E7 histidine in lowering oxygen affinity in the primordial globin was discussed. This is followed by the suggestion that rudiments of  $\alpha_1\beta_2$  contact sites were present in the monomeric vertebrate globin ancestor. This is based on the finding that identical amino acids at  $\alpha_1\beta_2$  contact sites, such as C2, FG3, FG5, G1 and G2 appear at all the branch points of the phylogenetic tree. The ancestor-descendent relationships of amino acids at functionally important sites further indicate that enhancement of cooperativity probably came about by the specific amino acid replacements that occurred after alpha-beta divergence at sites, such as C3, C5, C6, HC3 and FG4.

The experience gained from molecular genealogical studies on hemoglobin strengthened my conviction that biochemical systematics can be utilized to deduce phylogeny of living organisms. For this purpose, I spent my sabbatical year during 1979-80 at Georgetown University, USA, where I collaborated with Prof. Margaret Dayhoff to discern the major metabolic innovations in the Precambrian,

utilizing the information from the phylogenetic schema based on the sequences of bacterial ferredoxins, 2Fe-2S ferredoxins, 5S ribosomal RNA and c-type cytochromes<sup>22</sup>. These studies led to the identification of three major metabolic zones in the Precambrian. The middle zone, which reflects the genesis of oxygen-releasing photosynthesis and aerobic respiration, links metabolic innovations of the anaerobic stem on the one hand, and proliferation of aerobic bacteria and symbiotic associations leading to eukaryotes on the other. More specifically, *Halobacteria* and *Thermoplasma*, now known to belong to archaeobacteria, arise as separate branches from the eukaryote cytoplasmic line, indicating the polyphyletic origin of archaeobacteria.

During my tenure of the Jawaharlal Nehru Fellowship in 1983-85 at the National Chemical Laboratory (NCL), Pune, I have reviewed the available information, including our own, on evolutionary molecular biology and, in turn traced the outlines of evolution of three kingdoms of living organisms, namely, monerans, protists and animals<sup>23,24</sup>. In the discussion on the protistan phylogeny, ultrastructural, cytological and biochemical data were also examined. Against this background, I have also discussed the evolution of the chemistry of isoprenoids along with the diversity spectrum of carotenoids in plastids in relation to both algal phylogeny and endosymbiotic origins of plastids<sup>25</sup>.

As Head of the Division of Biochemical Sciences at NCL, the position which I took up in mid 1985, I have initiated programmes for computer-based molecular geneological analysis. Restriction mapping of mitochondrial DNA

is also currently being used by us as an evolutionary probe. Our attempts, I am sure, will provide us clearer insights into the understanding of the nature of diversity spectrum, order of deviation and relationships of living organisms.

For my contributions in the field of molecular evolution, I received the Shanti Swarup Bhatnagar award in 1974, and the Sreenivasayya memorial award in 1976.

### Selected Publications

1. Barnabas T, Badve M G & Barnabas J, Separation and identification of alkali metals by paper chromatography, *Naturwissenschaften*, **41** (1954) 478-79.
2. Barnabas T, Badve M G & Barnabas J, Chromatographic separation of mixture of the chlorides of calcium, strontium, barium and magnesium, *J Anal Chim Acta*, **12** (1955) 542-43.
3. Barnabas J & Joshi G V, Phenol-indo-2,6-dichlorophenol as a spray reagent, *Analyt Chem*, **27** (1955) 443.
4. Barnabas J & Muller C J, Hemoglobin Lepore Hollandia, *Nature, Lond*, **194** (1962) 931-32.
5. Ramakrishnan P & Barnabas J, Investigation on the heterogeneity of the verebrate hemoglobins, *J Acta Physiol Pharmacol Neerl*, **11** (1962) 328-42.
6. Ramakrishnan P & Barnabas J, Species similarity and variation in hemoglobins, *Indian J Biochem*, **4** (1967) 103-6.
7. Barnabas J, Comparison of homologous subunits of multiple hemoglobins from adult and new-born ruminants by peptide-pattern analysis, *Proc International Symposium on Comparative Hemoglobin Structure*, Thessaloniki, Greece, 1966, 29-35.
8. John M E & Barnabas J, Gene diversity of bovid hemoglobins, *Biochem Genet*, **16** (1978) 787-98.
9. Pratap P G, Nandi J & Barnabas J, Evolutionary trends in the hemoglobins of murine animals, *J Biosci*, **2** (1980) 369-78.
10. Balani A S & Barnabas J, Polypeptide chains of buffalo hemoglobins, *Nature, Lond*, **205** (1965) 1019-21.
11. Balani A S, Ranjekar P K & Barnabas J, Structural basis for genetic heterogeneity in the hemoglobins of adult and new-born ruminants, *Comp Biochem Physiol*, **24** (1968) 809-15.



12. Mawal R B, Barnabas T & Barnabas J, Identity of cow beta-lactoglobulin-B and buffalo beta-lactoglobulin, *Nature, Lond*, **205** (1965) 175-76.
13. Matsuda G, Maita T, Nakashima T, Barnabas J, Ranjekar P K & Gandhi N S, The primary structures of the alpha and beta polypeptide chains of adult hemoglobin of the Hanuman Langur (*Presbytis entellus*), *Int J Peptide Protein Res*, **5**(6) (1973) 423-25.
14. Barnabas J, Goodman M & Moore W, Evolution of hemoglobin in primates and other therian mammals, *Comp Biochem Physiol*, **39B** (1971) 455-82.
15. Goodman M, Barnabas J, Matsuda G & Moore W, Molecular evolution in the descent of man, *Nature, Lond*, **233** (1971) 604-13.
16. Barnabas J, Goodman M & Moore G W, Descent of mammalian alpha globin chain sequences investigated by the maximum parsimony method, *J molec Biol*, **69** (1972) 249-78.
17. Moore G W, Barnabas J & Goodman M, A method for constructing maximum parsimony ancestral amino acid sequences on a given network, *J theor Biol*, **38** (1973) 459-85.
18. Moore G W, Goodman M & Barnabas J, An iterative approach from the standpoint of the additive hypothesis to the dendrogram problem posed by molecular data sets, *J theor Biol*, **38** (1973) 423-57.
19. Barnabas J, Mathew P A, Ratnaparkhi M V & Barnabas S, Evolutionary genetics of mammalian hemoglobins and myoglobins during Cenozoic history, *Indian J Biochem Biophys*, **15** (1978) 388-400.
20. Barnabas J, Ratnaparkhi M V & Mathew P A, Molecular phylogeny of mammalian globin chain sequences deduced by using a parsimony probe, in *Biomolecular structure conformation, function and evolution*, Vol 2 (Pergamon Press, Oxford and New York) 1980, 635-42.
21. Furtado M, Mathew P A & Barnabas J, Evolutionary analysis of functional properties in hemoglobins, *Proc Indian natn Sci Acad*, **B47** (1982) 937-47.
22. Barnabas J, Schwartz R M & Dayhoff M O, Evolution of major metabolic innovations in the Precambrian, *Origins of Life*, **12** (1982) 81-91.
23. Barnabas S & Barnabas J, Aspects of biological evolution based on molecular data sets, *Proc Indian Acad Sci (Anim Sci)*, **93** (1984) 141-58.
24. Barnabas S & Barnabas J, Phylogenetic perspectives of protists, *Indian National Science Academy, Golden Jubilee Publications*, New Delhi (1984) 1-55.
25. Ladwa P H & Barnabas J, Evolutionary perspectives of isoprenoid chemistry, *Curr Sci*, **54** (1985) 111-18.

## Amar Nath Bhaduri

Bhaduri is one of the foremost enzymologists of India today. He had an early training in enzymology and metabolic biochemistry. As a doctoral candidate, he established the role of citrate-cleavage enzyme as the extramitochondrial source of acetyl CoA from citrate for the biosynthesis of fatty acids. During this work, Bhaduri also determined the stereospecificity of the cleavage enzyme with regard to its pro-chiral substrate. As a research fellow at Harvard Medical School in the group of Prof. H M Kalckar, Bhaduri further broadened his experience in enzymology and metabolic biochemistry. He returned to India in 1966, but could set up a small laboratory at the Department of Pharmacy of Jadavpur University only in the beginning of the seventies.

During the last 15 years, Bhaduri has studied intensively, at the molecular level, the catalytic mechanism, quaternary structure and regulatory potential of a model enzyme that catalyzes a reversible reaction of an amphibolic pathway and is obligatory for both the anabolic and the catabolic routes of metabolism. UDPglucose 4-epimerase in *Saccharomyces fragilis* catalyzes a freely reversible reaction between UDPglucose and UDPgalactose and is essential for both galactose degradation and biosynthesis of galacto-conjugates. The holoenzyme is composed of two apparently identical

subunits of mol wt 60,000. One mole of NAD is tightly bound to the dimer and generates a fluorophore (360 nm  $\rightarrow$  435 nm) on the enzyme surface. Mechanistically, the enzyme is an oxido-reductase and catalysis proceeds through enzyme-bound intermediates of UDP4-keto hexose and NADH. Thus, this epimerase is also the prototype of a large number of oxido-reductases. The presence of a single active site per dimeric apoenzyme raises interesting questions of protein assembly and symmetry at the active site.

Bhaduri and his group showed that the model enzyme could be completely inactivated and then reconstituted by several techniques, such as treatment with mercurials, treatment with a diazene dicarboxylic acid derivative that specifically crosslinks conformationally vicinal sulphydryl groups and by controlled heat treatment. Changes in quaternary structure during inactivation and on subsequent reconstitution were studied in each of these cases. With mercurials, the holoenzyme dissociated into monomers and free coenzyme. Reconstitution needed exogenous NAD and a thiol. On heat treatment, dimeric structure was retained, but the coenzyme dissociated. In contrast, inactivation on diamide treatment was exclusively due to the formation of a single disulphide bond across the subunits, even though the full holoenzyme structure was



retained. Further, in this case, an excellent correlation between loss of activity and loss of coenzyme fluorescence during inactivation and gain in these properties during reactivation was observed. These extensive studies on correlation between quaternary structure, enzyme activity and coenzyme fluorescence has provided new insights into the assembly process of a dimeric enzyme with a single active site.

The regulatory potential of this reversible enzyme has been indicated by extensive kinetic analysis. The enzyme shows an interesting asymmetry in kinetics with regard to its two substrates. Sigmoidal kinetics with one of the substrates and with sugar-phosphates as effectors are indicative of cooperative interactions at the subunit level.

In a major effort to understand the molecular mechanism of catalysis, the active site of the enzyme has been carefully mapped with specific modifying reagents by applying kinetic, differential spectrophotometric, fluorimetric and radiolabelling methods. Two conformationally vicinal sulphhydryl groups, each residing in separate subunits, have been located in the catalytic region of the active site. At least one histidine residue is located nearby and is probably involved as a prototrop in catalysis. The substrate-binding region contains two cationic arginine residues that are involved in the binding of the phosphate moiety of the substrate. The classic dinucleotide fold and a hydrophobic pocket for adenine moiety in NAD have been identified in the coenzyme binding region of the active site. These studies have revealed for the first time a tertiary structure homology at the active site between the family of oxido-

reductases and the more exhaustively studied dehydrogenases.

### Selected Publications

1. Bal D K & Bhaduri A, Nucleotide inhibition of UDPglucose 4-epimerase from *Saccharomyces fragilis* and from goat-liver, *Biochim biophys Acta*, **250** (1971) 588-91.
2. Ray M & Bhaduri A. UDPglucose 4-epimerase from *Saccharomyces fragilis*: Interaction with sugar phosphates at an effector site, *Biochem biophys Res Commun*, **60** (1974) 1081-89.
3. Ray M & Bhaduri A, Galactose 6-phosphate dehydrogenase purification and partial characterization, *J biol Chem*, **250** (1975) 3395-3401.
4. Ray M & Bhaduri A, UDPglucose 4-epimerase from *S. fragilis*: Allosteric kinetics with UDPglucose as substrate, *J biol Chem*, **250** (1975) 4373-75.
5. Ray M & Bhaduri A, UDPglucose 4-epimerase from *S. fragilis*: Desensitization with heat, *Biochem biophys Res Commun*, **67** (1975) 877-82.
6. Ray M & Bhaduri A, UDPglucose 4-epimerase from *S. fragilis*: Inactivation by heat and reconstitution of the inactive enzyme, *Eur J Biochem*, **70** (1976) 319-23.
7. Ray M, Kar K & Bhaduri A, UDPglucose 4-epimerase from *S. fragilis*: Involvement of sulphhydryl groups at the active site, *Biochem biophys Acta*, **526** (1978) 635-39.
8. Ray M & Bhaduri A, Presence of two conformationally vicinal sulphhydryl groups at the active site at UDPglucose 4-epimerase from *S. fragilis*, *J biol Chem*, **235** (1980) 10777-81.
9. Ray M & Bhaduri A, Fluorescence properties of reconstituted forms of UDPglucose 4-epimerase from *Saccharomyces fragilis*, *J biol Chem*, **235** (1980) 10782-86.
10. Samanta A & Bhaduri A, Presence of dinucleotide fold in UDPglucose 4-epimerase from *Saccharomyces fragilis*, *Biochem biophys Acta*, **707** (1982) 129-32.
11. Samanta A & Bhaduri A, Characterization of pyridine nucleotide binding site of UDPglucose 4-epimerase from *Saccharomyces fragilis*, *J biol Chem*, **258** (1983) 11118-22.
12. Mukherjee S & Bhaduri A, Presence of an arginine residue at the substrate binding site of UDPglucose 4-epimerase from *Saccharomyces fragilis*, *J biol Chem*, **261** (1986) 4519-24.

## P M Bhargava

### **(A) Pre-doctoral and Post-doctoral Work**

(1) *Relationship between structure and insecticidal activity*: In the late 1940's, Bhargava synthesized a series of new compounds and tested them for their insecticidal activity; in this way, he arrived at several significant conclusions regarding structure-activity relationships in this field. This work was done under the supervision of Dr A B Sen at Lucknow.

(2) *Studies in carcinogenesis*: In the mid-1950's, Bhargava studied, in the laboratory of Prof. Charles Heidelberger, the binding of carcinogenic hydrocarbons with proteins. Even though it was later found that the final conclusions were incorrect, the work opened up a new line of investigation in the field of cancer research. During this period, Bhargava was also involved in the work in Heidelberger's laboratory that laid the foundations for the discovery of fluorouracil.

### **(B) Independent Work**

(3) *Synthetic organic chemistry*: In the early 1950's, Bhargava and his coworkers worked out a new synthesis of analogues of stilbesterol and, in this process, discovered a new organic reaction. This work subsequently led to a new synthesis of stilbesterol by a group of Japanese workers.

(4) *Macromolecular metabolism of spermatozoa*: Bhargava and his colleagues were the first to study the macromolecular metabolism of spermatozoa. He and his coworkers have shown that transcription and translation in mammalian spermatozoa are confined to their mitochondria. This investigation has provided formal proof for the autonomy of mitochondrial transcription and translation.

(5) *Studies on a new set of proteins with unusual properties, isolated from seminal plasma*: Bhargava's group has recently isolated five new proteins from bovine seminal plasma and studied their properties. Three of them have been called 'seminalplasmin', 'seminal plasma ribonuclease' (RNAase SPL) and 'antiseminalplasmin'; the other two are proteases. All the five proteins have been purified to homogeneity. Seminalplasmin has an isoelectric point of 9.8. It is, on molar basis, one of the most potent antimicrobial agents known, active on a wide spectrum of microorganisms, including yeast. It seems to act by entering the cell and inhibiting specifically the synthesis of RNA; it also inhibits reverse transcription. Seminalplasmin was the fourth antimicrobial protein to be isolated from mammalian sources, and the second protein shown to enter microbial cells. It is the only known protein inhibitor of rRNA



transcription. Seminalplasmin has by now been extensively studied in several laboratories; its sequence is known and the gene for it has been synthesized. RNAase SPL has a molecular weight of 28,000, an isoelectric point of 9.8 and a hitherto undescribed spectrum of activity under certain specified conditions. It is, like RNAase A, specific for pyrimidines, but, unlike RNAase A, it shows high activity on double-stranded RNA. (There is, however, some doubt as of now whether or not RNAase SPL is identical with a previously described nuclease from bovine seminal plasma, RNAase BS1; this doubt has not yet been resolved.) Antiseminalplasmin nullifies the inhibitory effect of seminalplasmin on the growth of *E. coli* and may act by preventing the entry of seminalplasmin into the cells. The proteases—one acid and one neutral—also show unusual (hitherto undescribed) specificities; they are weakly active and give a limited digest containing generally only large fragments.

(6) *Primary cell suspensions*: In the 1960's, Bhargava and his colleagues started the first systematic investigation of primary cell suspensions obtained from solid tissues. They stated the importance and usefulness of these studies, worked out new methods for the preparation of the cell suspensions (specially liver and kidney cell suspensions), and studied the basic properties of the cell suspensions. The methods developed in Bhargava's laboratory were used widely and have since then been improved by others. This work served as the starting point for the development of this field (of primary liver cell suspensions) in which several hundred papers have appeared each year in the last ten years or so. Bhargava's group continues to be active in this area. Some

of the significant contributions of Bhargava's group in this field are as follows:

(a) *Methodology*: As stated above, Bhargava's group developed new methods for the preparation of liver (and kidney) cell suspensions.

(b) *Biochemical basis of intercellular organization*: A comparison of the properties of primary liver cell suspensions with those of the same cells organized in the tissue, made by Bhargava's group, has suggested that one of the purposes of intercellular organization may be to confer certain permeability characteristics on the cell membrane, which are altered on dispersion of the tissue to a single cell suspension.

(c) *Chemical and cellular composition of liver*: Bhargava's group was among the earliest to obtain reliable—and now widely used—quantitative information on several parameters concerning chemical and cellular composition of liver.

(d) *Ageing*: Bhargava's group showed, using interference microscopy of single liver cell in suspension, that as animals grow, the number of liver parenchymal cells per unit weight of liver decreases, the average cell size increases, and average density (dry weight per unit volume) decreases.

(e) *Cell recognition and specificity of liver cells*: In the mid-1960's, Bhargava's group showed that adult liver cells in suspension are able to recognize their own kind; when injected *in vivo*, they home to liver.

(f) *Aspects of structure and ribosomes*: In the mid-1960's, Bhargava's group also showed that liver ribosomes carrying

partially degraded RNA can retain their morphological and functional characteristics.

(g) *Uptake of nucleic acids by mammalian cells*: Bhargava's group was one of the earliest to carry out systematic studies in this important area. Even today, perhaps the most widely quoted review in this area is that of Bhargava and Shanmugam (1971). Recently, Sirdeshmukh and Bhargava have shown that a part of the RNA entrapped in liposomes is exposed.

(h) *Cell surface ribonucleases*: Bhargava's group has recently demonstrated the existence of a cell-surface ribonuclease in liver cells in suspension (and on bovine spermatozoa); these are among the first definitive demonstrations of cell-surface ribonucleases.

(7) *Regulation of cell division, growth and malignant transformation*: During the last several years, Bhargava and his colleagues have carried out extensive studies which provide strong support to the view that the control of uptake of essential nutrients plays a vital role in the regulation of cell division and malignant transformation in higher organisms. Several of these studies, such as those described in 1975-76 in a series of six papers in *Journal of Membrane Biology*, have been the first of their kind. On the basis of these studies, and an extensive analysis and evaluation of work done in areas related to the regulation of growth and malignant transformation, Bhargava proposed, in 1977, a new model for the regulation of growth and malignant transformation which has been widely discussed. New experimental support for this model has already been obtained in

Bhargava's laboratory, by the isolation, purification to homogeneity and studies on the location and the physical, chemical and biochemical properties of a new type of transport-inhibitory protein from rat liver that appears to satisfy the criteria (for which it has been tested) for such a protein predicted in the above-mentioned model. This protein appears to be modified in a chemically induced rat-liver tumour (the Zajdela ascitic hepatoma).

Bhargava was, perhaps, the first to demonstrate what is now known as the "cell concentration effect", a phenomenon which appears to be related to regulation of growth.

(8) *Mould metabolism*: In the early 1960's, Bhargava and his colleagues made the interesting observation that pyruvate reverses the inhibition by fluoride of acid production and growth in an itaconic acid-producing strain of *Aspergillus terreus*.

(9) *The second genetic code*: In 1971, Bhargava and two of his colleagues argued in favour of the existence of a second genetic code in tRNA. This argument attempted to provide a rationale for the specificity of the interaction between tRNA and aminoacyl-tRNA synthesis. Although their hypothesis has by no means been proven, the evidence that has accumulated over the years seems to be in consonance, in principle, with the above view.

(10) *The origin of life*: Bhargava has recently argued that DNA may not contain all the information for certain aspects of organization found in a cell, and that the problem of the origin of the first cell (transition from the chemical to the biological evolution) may essentially be a problem of the emergence of such organization; attempts are being made to verify these premises through



appropriately designed experiments and theoretical model building.

(11) *How does a cell mark its proteins destined for degradation?* A new ATP and  $Mg^{2+}$ -dependent protease that may be involved in the ubiquitin-aided degradation of proteins in the cells has been purified to homogeneity from rabbit reticulocytes, and its properties studied by Bhargava's group.

(12) "Restriction-type" ribonucleases: The existence of a ribonuclease, which degrades *E. coli*, *B. subtilis*, rat-kidney and rabbit-liver rRNAs but not rat-liver rRNA, has been demonstrated in rat liver, and its specificity and properties studied recently by Bhargava and his colleagues.

### Selected Publications

1. Bhargava P M & Sen A B, Search for insecticides: Chemical constitution and insecticidal activity, *J Sci Fd Agric*, **1** (1950) 178-82.
2. Bhargava P M & Zaheer S H, A new synthesis of some derivatives of 4,4'-stilbenediol, *Nature, Lond*, **171** (1953) 746-47.
3. Jacob S T & Bhargava P M, A new method for the preparation of liver cells in suspension, *Exp Cell Res*, **27** (1962) 453-67.
4. Iype P T, Bhargava P M & Tasher A D, Some aspects of the chemical and cellular composition of adult rat liver, *Exp Cell Res*, **40** (1965) 233-51.
5. Bhargava P M & Shanmugam G, Uptake of non-viral nucleic acids by mammalian cells, in *Progress in Nucleic Acid Research and Molecular Biology*, Vol II, edited by W Cohn and J N Davidson (Academic Press, New York), 1971, Chap IV, 103-91.
6. Bhargava M, Siddiqui M A, Kranti Kumar G & Prasad K S N, Effect of cell concentration on the uptake of essential aminoacids by rat-liver parenchymal cells in suspension, *J Memb Biol*, **22** (1975) 357-68.
7. Bhargava P M, Allin E P & Montagnier L, Uptake of aminoacids and thymidine during the first cell cycle of synchronised hamster cells, *J Memb Biol*, **26** (1976) 1-17.
8. Bhargava P M, Regulation of cell division and malignant transformation. A new method for control by uptake of nutrients, *J theor Biol*, **68** (1977) 101-37.
9. Bhargava P M, Incorporation of radioactive aminoacids into the proteins of bull spermatozoa, *Nature, Lond*, **179** (1957) 1120-21.
10. Premkumar E & Bhargava P M, Transcription and translation in bovine spermatozoa, *Nature (New Biol), Lond*, **240** (1972) 139-43.
11. Reddy E S P & Bhargava P M, Seminalplasmin: An antimicrobial protein from bovine seminal plasma which acts in *E. coli* by specific inhibition of rRNA synthesis, *Nature, Lond*, **279** (1979) 725-28.
12. Scheit K H, Reddy E S P & Bhargava P M, Seminalplasmin is a potent inhibitor of *E. coli* RNA polymerase *in vitro*, *Nature, Lond*, **279** (1979) 728-31.
13. Rao Veena N & Bhargava P M, Isolation, characterisation and the mechanism of action of antiseminalplasmin—A new protein that inhibits the antimicrobial activity of seminalplasmin, *Biochem J*, **227** (1985) 609-19.
14. Bhargava P M, Pallaiah T & Premkumar E, Aminoacyltransfer R N A synthase recognition code-words in yeast transfer RNAs: A proposal, *J theor Biol*, **29** (1970) 447-69.
15. Bhargava P M, The "what" and "why" of biochemistry, in *Textbook of biochemistry and human biology*, edited by G P Talwar (Prentice-Hall, New Delhi) 1980.

## B B Biswas

### (1) Nucleic Acid Research

In the first phase of his research career, Biswas contributed substantially on nucleic acids of blue green algae and analyzed the base composition of DNA from *Nostoc muscorum*<sup>1</sup> and *Anacystis nidulans*<sup>2,3</sup>. This work constituted a part of his doctoral thesis and it is now a classical reference for any textbook on blue green algae. He continued this aspect of work while he was in USA. He was one of the scientists who discovered RNA polymerase<sup>4,5</sup> and methylation of RNA<sup>6</sup> while working at Pittsburgh, USA. Later, he continued the work on ribopolynucleotide synthesis in chloroplasts<sup>7,8</sup> and control of the transcription process, particularly in plant cells and discovered three RNA polymerases, one for messenger RNA, the second for ribosomal RNA and the third one for tRNA synthesis along with a few regulatory factors from the chromosomal nonhistone proteins<sup>9,10</sup>. That a plant growth substance such as IAA can act as a modulator of the transcription process has also been elucidated<sup>11,12</sup>. A membrane-bound receptor for IAA might exert an early action on the regulation of synthesis of the high affinity receptor for auxin<sup>13</sup>.

### (2) Establishment of Metabolic Cycle in Plants Involving Myoinositol Phosphates

While elucidating the role of myoinositol hexaphosphate ( $IP_6$ ) in the regulation of

phosphorus metabolism in the seeds, a novel metabolic cycle involving glucose-6-P and myoinositol phosphates during formation and germination of seeds was discovered by Biswas and his associates. This is a novel metabolic cycle proposed for the utilization of the phosphorus reserve material, viz.  $IP_6$ , in the seeds. It is now well established that during formation and maturation of seeds, glucose-6-P is diverted to synthesize myoinositol-1-P, which is subsequently transformed into myoinositol hexaphosphate by phosphoinositol kinase<sup>14</sup> and  $IP_6$ -ADP phosphotransferase<sup>15</sup>. When the seed germinates, one of the earliest events is the transfer of phosphoryl group of  $IP_6$  to ribonucleoside-diphosphates to synthesize either ATP or GTP and  $IP_5$  by phosphotransferase. Subsequently, this  $IP_5$  (20H) is degraded to the penultimate product of myoinositol-1-P by phytase<sup>14</sup>. This myoinositol-1-P is further converted to Ru-5-P by myoinositol-1-P-dehydrogenase through oxidation and decarboxylation reactions, and cycled back to glucose-6-P. In its oxidation, NAD is reduced to NADH. Thus, both ATP and the reducing power, i.e. NADH, are generated during germination of seeds. Since Ru-5-P can enter into the pentose shunt pathway and Calvin's cycle for photosynthetic fixation of  $CO_2$ , this novel metabolic cycle seems to play an important role during the early phase of



germination<sup>16-18</sup>. The future research will include (i) raising certain mutants of mung bean plants defective in particular enzymes of this cycle in order to study the involvement of this cycle in early germination or seedling vigour; (ii) the involvement of NADH as a source of early ATP generation in the germination of seeds or in other cell systems; (iii) the efficiency of photosynthesis involving myoinositol-1-P dehydrogenase in the leaves/chloroplast; and (iv) generation of myoinositol triphosphates (1,4,5-IP<sub>3</sub>) as second messenger for the development of plant cell. The gene for the enzyme myoinositol-1-P-dehydrogenase is now being cloned using recombinant DNA technique to study the regulation of that gene.

### (3) Molecular Biology of Tubulin: Its Interaction with Drugs and Genomic Organization

The binding of colchicine with tubulin is one of the primary characteristics of the protein and colchicine binding has been studied with tubulin prepared from many organisms and the higher plants. The chemical specificity of the colchicine-binding site of tubulin is less stringent for the presence of the B-ring than the A- and C-rings of colchicine. Colchicine analogues with modifications in the B-ring bind to tubulin at the same site as colchicine. Analogues with smaller or no substituents in the B-ring bind tubulin remarkably faster than colchicine. Colchicine and 2-methoxy-5 (2,3,4-trimethoxyphenyl) tropone bind reversibly to tubulin, whereas colchicine and desacetamidocolchicine bind almost irreversibly, suggesting that the size of the B-ring moiety of colchicine is not related to the reversibility of binding<sup>19,20</sup>. Further, it has been shown

that tubulin has two distinct colcemid binding sites. One site has a very high affinity, while the other has low affinity<sup>21</sup>. The activation energy of the colcemid binding to tubulin has been found to be 9.8 kcal/mol, a value lower than that for colchicine, 19.5 kcal/mol<sup>22</sup>. It is also revealed that there is a vast difference in the activation energy and rate of binding among colchicine and its B-ring analogues. It has also been observed that colchicine can bind with polysomes isolated from rat brain<sup>23</sup>. Tubulin mRNA thus enriched from polysomal fraction was used for cDNA synthesis and cloning work. Both the subunits of plant tubulin have been found to be different from those of the brain. The affinity of plant tubulin towards colchicine has been found to be 1-2 orders of magnitude lower than that found with animal tubulin<sup>24</sup>. Both the genes for  $\alpha$  and  $\beta$  subunits of plant (*Vigna radiata*) tubulin have recently been cloned in *E. coli* and their expression in *E. coli* is being followed. Plant tubulin genes are closely associated and tandemly arranged<sup>25</sup>. Plant tubulin binding with certain pesticides and insecticides is being standardized to evolve an assay for providing a guideline for using specific pesticides. The heterogeneity of plant tubulins and their coordinate and regulation of expression forms another facet of the work. Considering that nuclear matrix, nuclear envelope and cytoskeletal elements might be involved in maturation, transport and decay of mRNAs, in general, it would be interesting to find out any interaction of these components with the autoregulatory control of tubulin mRNA synthesis. Several pseudogenes of tubulin have been reported from different systems. Since the pseudogenes of tubulin retain many of the features of expressed genes, it is possible that the generation of

new functional sequences might arise out of them. This is to be tested.

The programme of recombinant DNA work is now being directed to clone some of the genes for surface proteins and tubulins of *Antamoeba histolytica*, causing gastrointestinal disorder.

### Selected Publications

1. Biswas B B, Chemical nature of nucleic acids in Cyanophyceae, *Nature, Lond*, **177** (1956) 95.
2. Biswas B B & Myers J, Characterization of DNA from *Anacystis nidulans*, *Nature, Lond*, **188** (1960) 1029.
3. Biswas B B & Myers, J, A methyl cytidine from RNA of *Anacystis nidulans*, *Nature, Lond*, **186** (1960) 238.
4. Abrams R, Edmonds M & Biswas B B, Polynucleotide synthesis from nucleoside triphosphates with enzymes of calf thymus nuclei, *Proc Symposium on ribonucleic acids and polyphosphates: Structure, synthesis and function*, Strassburgh (CNRS, Paris) 1961, 323.
5. Biswas B B & Abrams R, Enzymatic incorporation of nucleoside triphosphates into nuclear RNA, *Fedn Proc*, **20** (1961) 362; *Biochim biophys Acta*, **55** (1962) 827.
6. Biswas B B, Edmonds M & Abrams R, Methylation of purines of soluble ribonucleic acid with  $C^{14}$  methyl methionine, *Biochem biophys Res Commun*, **6** (1961) 146.
7. Chakraborty A K & Biswas B B, Nonterminal incorporation of guanosine monophosphate from guanosine triphosphate by chloroplast extract, *J biol Chem*, **240** (1965) 4406.
8. Biswas S & Biswas B B, Nonterminal incorporation of adenosine monophosphate from adenosine triphosphate by chloroplast extract, *Archs Biochem Biophys*, **114** (1966) 472.
9. Mondal H, Mandal R K & Biswas B B, RNA polymerase from Eukaryotic cells. I. Isolation and purification of enzymes and factors from the chromatin of coconut nuclei, *Eur J Biochem*, **28** (1972) 463.
10. Mondal H, Ganguly A, Das A, Mandal R K & Biswas B B, RNA polymerase from Eukaryotic cells. II. Effects of factors and Rifampicin on the activity of RNA polymerase, *Eur J Biochem*, **28** (1972) 143.
11. Mondal H, Mandal R K & Biswas B B, RNA stimulated by indole acetic acid, *Nature (New Biol)*, **240** (1972) 111.
12. Biswas B B & Roy P, Plant growth substances as modulators of transcription, *Sub cell Biochem*, **5** (1978) 187.
13. Bhattacharyya K & Biswas B B, Induction of a high affinity site for auxin in *Avena* root membrane, *Phytochemistry*, **21** (1982) 1207.
14. Lahiri Majumder A, Mandal N C & Biswas B B, Phosphoinositol kinase from germinating mung bean seeds, *Phytochemistry*, **11** (1972) 503.
15. Biswas S, Maiti I B, Chakraborty S & Biswas B B, Purification and characterization of Myoinositol-hexa-phosphate-ADP-phosphotransferase from *Phaseolus aureus*, *Archs Biochem Biophys*, **185** (1978) 557.
16. De B P & Biswas B B, Evidence for the existence of a novel enzyme system, Myoinositol-1-P dehydrogenase in *Phaseolus aureus*, *J biol Chem*, **254** (1979) 8717.
17. Ghosh B, De B P & Biswas B B, Purification and properties of Myoinositol-1-phosphate dehydrogenase from germinating mung bean seeds, *Archs Biochem Biophys*, **228** (1984) 309.
18. Biswas B B, Ghosh B & Lahiri-Majumder A, Myoinositol polyphosphates and their role in cellular metabolism: A proposed cycle involving glucose-6-P and myoinositol phosphates, *Sub Cellular Biochem*, **10** (1984) 237.
19. Ray K, Bhattacharyya B & Biswas B B, Role of B-ring of colchicine in its binding to tubulin, *J biol Chem*, **256** (1981) 6241.
20. Biswas B B, Banerjee A C & Bhattacharyya B, Tubulin and microtubule system in cellular growth and development, *Sub Cell Biochem*, **8** (1981) 123.
21. Ray K, Bhattacharyya B & Biswas B B, Anion-induced increases in the affinity of colcemid binding to tubulin, *Eur J Biochem*, **142** (1984) 577.
22. Ghosh Choudhury G, Banerjee A, Bhattacharyya B & Biswas B B, Interaction of colchicine analogues with purified tubulin, *FEBS Lett*, **161** (1983) 55.
23. Chakrabarti S, Sen K, Bhattacharyya B & Biswas B B, Colchicine binding activity of polysomes, *J Biosci*, **5** (1983) 203.
24. Biswas B B, Sen K, Ghosh Choudhury G & Bhattacharyya B, Molecular biology of tubulin: Its interaction with drugs and genomic organization, *J Biosci*, **6** (1984) 431.
25. Raha D & Biswas B B, Cloning of  $\beta$ -tubulin cDNA and its organization in *Vigna radiata* genome, *Plant molec Biol*, (1986), in press.



## D P Burma

Burma started his research career in 1949 as a physical chemist and practically with a drain pipe. In those days, sophisticated techniques were hardly used in the country and the technique of paper chromatography was something new. Therefore, he had to prepare his own chromatographic chamber (the first version was a drain pipe) to study the mechanism of separation of various materials on filter paper. The main finding that emerged from the extensive studies conducted is as follows. Although the basic mechanism of separation as established by the discoverers of the technique (Martin and Synge) who eventually got the Nobel Prize was essentially right, the process of absorption on the filter paper is also partially responsible for the separation other than the partition between the two phases created on the paper<sup>1,2</sup>. Of the numerous papers published in that area, the major one which is quoted in almost all textbooks on paper chromatography was in Analytical Chemistry<sup>2</sup>. The next step was to use the technique along with the technique of paper electrophoresis in the separation of various types of compounds which drew his attention to the fantastic work that was then being done by Melvin Calvin. He used the technique in conjunction with radioactive tracer technique for the solution of the problem

of photosynthesis in plants and eventually got the Nobel Prize for this work.

Although Burma was a physical chemist by training and had no formal training in biology, he had close association with a number of biologists at the Bose Institute where he started his career. The strong desire of the Director of the Institute, late Dr D.M. Bose, who was the nephew of Sir J C Bose and was always keen to solve the problems studied by his uncle, led Burma to proceed abroad in 1954 to study the mechanism of photosynthesis in plants. A fellowship obtained from the National Research Council, Canada, enabled him to spend one year in the NRC laboratory at Ottawa studying the mechanism of synthesis of sugar in sugarbeet leaves. He could demonstrate that sucrose is synthesized in these leaves from uridine diphosphate glucose<sup>3</sup>. Melvin Calvin had already demonstrated this, but by a somewhat different pathway in chlorella.

From Canada he moved to the University of Wisconsin at Madison, USA to work with Prof. R H Burris in an entirely different area. On the one hand he was keen to learn the technique of mass spectrometry, on the other he was trying to understand the mechanism of fixation of nitrogen by the organism *Azotobacter vinelandii* in which Dr Burris was deeply involved. He had earlier gained some

experience at the Bose Institute on the fixation of nitrogen in rhizobium legumes. That ammonia is a key intermediate in nitrogen fixation could be demonstrated using  $^{15}\text{N}$  as tracer<sup>4</sup>. He could also demonstrate the flow of ammonia to various nitrogenous compounds, mainly amino acids and bases. This work eventually led him to demonstrate the incorporation of amino acids into proteins in cell-free preparations of *Azotobacter vinelandii*. Finally, he moved into the laboratory of Prof. Bernard L Horecker, an internationally known enzyme chemist. (Incidentally, the 70th birthday of Horecker was celebrated recently with a symposium in which Burma gave an invited talk.) Not only that he got the chance of a very fine training in enzyme chemistry under Horecker's direct supervision, but he was able to discover two new enzymes involved in the metabolism of pentoses by *Lactobacillus pentosus*<sup>5</sup>. The two enzymes, L-ribulokinase and L-ribulose-5-phosphate-4-epimerase, are involved in the metabolism of L-arabinose.

After three years of post-doctoral training and research, Burma returned to the Bose Institute in 1957 and started his own work in biochemistry. At that time, his would-be wife, Maharani Chakravorty, joined him as his PhD student. The main attempt was to demonstrate the pentose phosphate pathway in plants. All the enzymes of the pathway could be demonstrated in mung-bean seedling. Another approach was in the field of protein synthesis which was in a very early stage at that time<sup>6</sup>. Due to some institutional problems, he had to again leave for USA and this time chose the laboratory of Prof. Severo Ochoa, a Nobel Laureate. Although Ochoa got the Nobel

Prize for demonstrating the synthesis of RNA in a test tube, his enzyme polynucleotide phosphorylase could not synthesize RNA under *in vivo* condition. Several laboratories were competing with one another to discover the new enzyme and it was practically horse-racing. Finally, all the laboratories, including Ochoa's<sup>7</sup>, came with the same answer with the discovery of the enzyme RNA polymerase which catalyses the synthesis of RNA on DNA template. The discovery of this enzyme linked up the genetic machinery with the proteins, the products of the genes.

After his return to the Bose Institute in 1961, he devoted himself primarily to the study of the enzymes involved in the metabolism of RNA. He could also show the occurrence of an interesting enzyme in *Azotobacter vinelandii*, which catalyses the addition of guanine bases at the end of RNA chains<sup>8</sup>.

When he joined the Banaras Hindu University in 1964, he had practically no laboratory, no equipment and no facilities for research. It took him quite some time to build up the laboratories. Therefore, he had to work temporarily in a somewhat different area in spite of no expertise in that field. Maharani Chakravorty, who moved with him to Banaras, had an extensive training abroad in genetics and even the finest experiments of genetics could be done without the help of sophisticated equipment. Her major interest was in the virus p22. She had studied extensively the genetic characters of this virus abroad as well as in this country. Utilizing her experiences, Burma tried to study a little bit of biochemistry of the same virus. He was the first one to demonstrate the enzyme lysozyme in p22-



infected *Salmonella typhimurium*<sup>9</sup>. This enzyme was quite distinct in its properties from another temperate phage  $\lambda$ . On the other hand, it was very close in its properties to the lysozymes produced by the lytic phages. Later, it was demonstrated in another laboratory that  $\lambda$  phage does not actually produce a lysozyme but a neuraminidase. With the development of research facilities in the laboratory, he could gradually move into his own field, i.e. the metabolism of nucleic acids. His primary object at that time was to find out the enzyme which is responsible for the degradation of messenger RNA under *in vivo* conditions. With that objective in view, he started purifying a number of enzymes, RNases I, II and III and characterized one of them thoroughly<sup>10</sup>. None of them, however, proved to be responsible for the degradation of messenger RNA. However, the study of the enzyme RNase I led him to his present research interest in the field of 'Ribosomology'. It appeared during the purification of RNase I from *E. coli* that there was a strong inhibitor of the enzyme in the extract of *E. coli*. That inhibitor eventually turned out to be ribosome<sup>11</sup>. Since this enzyme has the peculiar characteristic of associating with the smaller subunit of the ribosome, it was used extensively in this laboratory to understand the differences in the structural organizations of the two subunits of *E. coli* ribosome. The major information obtained was that the larger subunit has a more flexible structure than the smaller one; at least some of the regions of the larger subunit, specially the L7/L12 stalk region, are more amenable to attack by this enzyme. This early work<sup>11-13</sup> in the ribosome field has been reviewed extensively by him. Simultaneously, work was started on the ribosomal RNAs free of

ribosomal proteins and that also with the help of RNase I. Immediately, the differences in the conformations of 16S and 23S RNAs were revealed and it was also realized that under the reconstitution condition of Nomura, both of them become resistant to the attack of this enzyme. Actually, these preliminary studies led to the demonstration for the first time in this laboratory that 16S and 23S RNAs form a specific bimolecular complex under two well-defined conditions, reconstitution condition as well as in the presence of alcohol<sup>14</sup>. Vasiliev and his co-workers in Russia had earlier demonstrated that ribosomal RNAs assume structural features of ribosomal subunits in the presence of alcohol. The small molecular weight RNA (5S), however, could not be incorporated into the complex and three specific proteins were required for the purpose<sup>15</sup>. The next step was to demonstrate whether such a complex has any biological activity. It could be demonstrated unequivocally that the complex displays a weak biological activity with a limited number of ribosomal proteins and the requisite factors added. The various steps of protein synthesis as well as polyphenylalanine synthesis could be demonstrated<sup>16</sup>. This has opened up a new area of investigation and is being pursued vigorously in order to understand the functions of proteins in building up centres of activity of the RNA-RNA complex. It is strongly believed that ribosomal RNAs are capable of carrying out the steps of protein synthesis and the proteins act as switches to give it the capacity to undergo the conformational change. Thus, it appears that ribosomal RNAs acted as primitive ribosomes, as suggested by Crick and Orgel. However, one has to go a long way to establish the hypothesis. There has been another

interesting turn with the observation that the larger subunits are capable of occurring in two conformations, tight and loose. Tight and loose couple 70S ribosomes were known for more than two decades; even it was known that the difference lies in the 50S subunit. But it has been demonstrated for the first time in this laboratory that this difference is due to the difference in the conformation of 23S RNA<sup>17</sup>. This is actually the result of the process of translocation, because the translocating agents like EF-G and GTP are capable of converting one form to the other. The translocation process is also known for quite a long time. It was thought to be due to the conformational change of either one or both the subunits, but no direct evidence was available from any of the laboratories. It has been demonstrated for the first time in this laboratory that the conformational change of 23S RNA in 50S ribosome is responsible for translocation. In order to understand the conformational change, attention has now been focussed on the regions of 23S RNA in which this change takes place. Preliminary evidences indicate that the 17/L12 stalk region may be one of the primary sites. Actually, several new vistas of research have opened up in molecular biology from the recent findings in his laboratory<sup>18,19</sup> and the very recent concept that RNAs may have catalytic functions is fully supported by these investigations. Ribosome in all its complexities seems to be the best material to prove the structure-function relationship in biological macromolecules.

### Selected Publications

1. Burma D P, Effect of the temperature on the Rf values of the amino acids during paper chromatography with solvents completely miscible with water. *Nature, Lond*, **168** (1951) 565.
2. Burma D P, Partition mechanism of paper chromatography with reference to absorption of chromatographed substances. *Analyt Chem*, **25** (1953) 549.
3. Burma D P & Mortimer D C, The biosynthesis of uridine diphosphate glucose and sucrose in sugar beet leaf, *Archs Biochem Biophys*, **62** (1956) 16.
4. Burma D P & Burris R H, Kinetics of ammonia utilization by *Azotobacter vinelandii*, *J biol Chem*, **225** (1957) 287; Metabolism of nitrogen by cell-free preparations from *Azotobacter vinelandii*, *J biol Chem*, **225** (1957) 723.
5. Burma D P & Horecker B L, Pentose fermentation by *Lactobacillus plantarum*, III. Ribulose 5-phosphate 4-epimerase, *J biol Chem*, **231** (1958) 1053.
6. Chakravorty M & Burma D P, Studies on the microbial protein synthesis with *Azotobacter vinelandii* as the test organism. I. Incorporation of radioactive phosphate and sulfate into the resting cells in the presence of antibiotics and antimetabolites, *Biochim biophys Acta*, **55** (1962) 110; II. Incorporation of radioactive amino acids into protein of the cell-free particulate preparations, *Biochim biophys Acta*, **55** (1962) 120.
7. Oacha S, Burma D P, Kroger H & Weil J D, Deoxyribonucleic acid-dependent incorporation of nucleotides from nucleoside triphosphates into ribonucleic acid, *Proc natn Acad Sci, USA*, **47** (1961) 670; Further studies on deoxyribonucleic acid-dependent enzymatic synthesis of ribonucleic acid, *Proc natn Acad Sci, USA*, **47** (1961) 749.
8. Majumdar C & Burma D P, DNA-dependent incorporation of guanylate residues into polynucleotide material, *Biochem Z*, **342** (1965) 410.
9. Koteswara Rao G R & Burma D P, Purification and preparation of bacteriophage induced lysozyme, *J biol Chem*, **246** (1971) 6474.
10. Chakraburttty K & Burma D P, Purification and properties of a ribonuclease from *Salmonella typhimurium* extracts, *J biol Chem*, **243** (1968) 1135.
11. Dutta A K & Burma D P, Association of RNase I with the ribosomes and their subunits, *J biol Chem*, **247** (1972) 6795.
12. Raziuddin, Chatterji D, Ghosh S & Burma D P, Site of action of RNase I on the 50S ribosome of *Escherichia coli* and the association of the



- enzyme with the partially degraded subunit, *J biol Chem*, **254** (1979) 10575.
13. Byasuni & Burma, D P, Structural alteration of rRNA in the L7/L12 region of 50S ribosome on removal of L7/L12 proteins. *Biochem biophys Res Commun*, **104** (1982) 99.
  14. Burma D P, Nag B & Tewari D S Association of 16S and 23S ribosomal RNAs to form a bimolecular complex. *Proc natn Acad Sci, USA*, **80** (1983) 4875.
  15. Tewari D S & Burma D P, Incorporation of 5S RNA into 16S. 23S RNA complex, *Biochem biophys Res Commun*, **114** (1983) 348.
  16. Burma D P, Tewari, D S & Srivastava, A K, Ribosomal activity of the 16S.23S RNA complex, *Archs Biochem Biophys*, **239** (1985) 427.
  17. Burma D P, Srivastava, A K, Srivastava S, Tewari D S, Dash D & Sengupta S K, Differences in physical and biological properties of 50S ribosomes and 23S RNAs derived from tight and loose couple 70S ribosomes, *Biochem biophys Res Commun*, **124** (1984) 970.
  18. Burma D P, Srivastava A K, Srivastava S & Dash D, Interconversion of tight and loose couple 50S ribosomes and translocation in protein synthesis, *J biol Chem*, **260** (1985) 10517.
  19. Burma D P, Srivastava S, Srivastava A K, Dash D & Mahanti S, Conformational change of 50S ribosomes during protein synthesis in *Ribosomes, structure, function and genetics*, edited by B Hardesty (Springer Verlag) 1986, in press.

## N N Das Gupta

The great importance of the development of Biophysical Science in India was foreseen, as early as 1940, by the late Prof. Meghnad Saha, FRS. Under his inspiration, a small nucleus for biophysical work was created in a small room in the University College of Science, Calcutta University, in 1944. The work that started with four research workers, expanded rapidly and the available space soon proved inadequate. The group had to shift in 1950 to the newly established Institute of Nuclear Physics. This is a short account of the work done here for the development of the science of Biophysics at Calcutta.

### **Biophysical Techniques and Instruments**

From the beginning, great stress was placed on the development of biophysical techniques and instruments, without which no experimental work was possible. The project on the construction of an electron microscope was started in 1944. This instrument, developed in Germany, was then not available commercially. It was decided to build an instrument in Calcutta. Fortunately, Prof. L Marton, one of the pioneers in this field, was then working as a refugee scientist in USA. He was approached to help Calcutta University in its project to build an electron microscope, which he kindly agreed to do. Accordingly, I spent the academic year 1945-46 with Prof. Marton in Stanford University,

California. With Prof. Marton's help, the construction of the vital components of the instrument was completed in USA. High voltage supply for the electron gun, the microscope filament supply, the current regulators for the lenses, the vacuum gauge and relay circuits were built up later at Calcutta. The completed instrument was installed in the University College of Science in 1948. This was the first electron microscope in India<sup>1</sup>.

Many other biophysical techniques were also developed here. These included the use of radioactive tracers in the investigations on biological and medical problems, ultrathin sectioning for electron microscopy, autoradiography, and ultracentrifugal and dielectric methods for studies on biological molecules.

### **Electron Microscopic and Tracer Research**

Immediately after the construction of the new microscope, many electron optical problems, e.g. the enhancement of contrast in the image and the effects of shadowing, staining and sectioning on the electronic image had to be investigated carefully.

At that time, many of the new facilities created in the biophysics laboratory were not available to scientists working in other research institutions in Calcutta. It was natural that a few of them wanted to share

---

Formerly, Sir Tarak Nath Palit Professor & Head, Department of Physics, Calcutta University, Calcutta; Residence : 44 Hazra Road, Calcutta-700019.



these facilities for their own work. The biophysics group collaborated with them as far as possible. The following electron microscopic studies were made during this initial period. Electron and phase microscopic studies were made on the nuclear apparatus of *Escherichia coli*. This showed contrast differences in the cytoplasmic and nuclear zones, which varied with the age of bacteria<sup>2</sup>. A new method was developed for electron microscopic examination of intracellular parasite *Plasmodium berghei* (malaria)<sup>3</sup>. The complex morphological structure of the leptomonad form of *Leishmania donovani* (Kalaazar protozoa) was the subject of a series of studies<sup>4</sup>. In collaboration with the School of Tropical Medicine, a detailed investigation was made on the electron microscopic appearance of *Mycobacterium leprae* obtained from patients who had not received any treatment and from patients under treatment with the drug DDS for over a year<sup>5</sup>.

Similar collaboration also developed between the biophysics group and Chittaranjan Cancer Hospital, where I was requested to help in the organization of tracer research in medical problems. The following investigations were made in this institution : (a) Measurement of the uptake of radioactive phosphorus by different tissues, (b) Differentiation between normal and malignant tissues on the basis of the uptake of radioactive phosphorus<sup>6</sup>, and (c) Assessment of thyroid function with a tracer dose of radio-iodine<sup>7</sup>. In another research, carried out in the biophysics laboratory in collaboration with Calcutta Medical College, the life-span of the circulating granulocytes in chronic leukemia was estimated. For this purpose, the rate of

incorporation of radioactive phosphorus in the DNA of the circulating leucocytes and its subsequent decrease had to be measured<sup>8</sup>.

These researches proved that the use of the latest physical methods and techniques could be highly fruitful in the solution of practical medical problems. There was great appreciation of the importance of the Science of Biophysics. Biophysics laboratories developed later in Chittaranjan National Cancer Research Centre and also in the School of Tropical Medicine, Calcutta, where the persons trained earlier were in charge for some years. In 1955, CSIR appointed a special committee in biophysics with Dr B C Roy as chairman. This committee recommended liberal grants to the institutions which had then started to work in this field. This gave further impetus to the development of biophysical science in India.

### Post-graduate Training in Biophysics

It was felt that for the proper development of this interdisciplinary science in addition to research facilities in the subject, there should also be a provision for systematic training of young graduates. There was no provision for this in any university in India at that time. A programme on post-graduate teaching in biophysics was introduced in Calcutta University in 1956. It was aimed to give special training in the theories and techniques frequently needed in the solution of problems in biology and medicine. Practical work consisted of the use of electron microscope, tracer technique, analytical ultracentrifuge and UV spectrophotometer in a few typical experiments. After passing MSc with specialization in biophysics, a few of the

students carried on post-MSc research on particular biophysical problems until they qualified for PhD degree. Some of the successful candidates have now been re-employed in the parent department, while a few others are heading biophysics departments in different universities and national laboratories. A good school in biophysics ultimately developed in Calcutta through the combined efforts of all of them.

### **Advancement of Electron Microscopy in India**

By 1962, several institutions in India were using electron microscopes in their work. An Electron Microscope Society of India was inaugurated in that year. Annual conferences of the society were held in different cities of India where electron microscopic work was going on. Two international conferences in electron microscopy were also organized: (a) The Far East and Oceanic Congress in Electron Microscopy (1965), and (b) The International Conference on Electron Microscopy in Life Sciences (1969). Prof. E Ruska, the discoverer of the electron microscope, and many other distinguished electron microscopists from Europe, Japan and USA took part in these conferences.

### **Researches in Molecular Biophysics**

Much of my later work has been concerned with studies on biological macromolecules with different techniques. A few of these are described below.

It is known that the human haemoglobin variants HbA, HbF and HbE differ in their primary structure. Such differences often lead to differences in the surface structure, charge, size and shape of a molecule. An attempt was, therefore, made to explore if

any difference could be detected in these molecules with the techniques of ultracentrifugation and high resolution electron microscopy. It was found that while HbA and HbE molecules had almost identical sedimentation constants (*S*), HbF showed higher *S* values. Under the electron microscope, most of the molecules had a pear shape, which seemed to be divided into unequal segments with depressions radiating from the centre. The centre also showed a big depression in some projections. The average dimensions of the stained molecules were about  $76\text{\AA} \times 63\text{\AA}^{9,10}$ .

The physical nature of the denatured and the renatured DNA molecules was the subject of a series of studies. Although hyperchromicity was produced in each case of denaturation, characteristic differences were noticed in DNA denatured by exposure to low molar solvents, by heat and by X-radiation. In the case of storage in low molar solvents, denatured DNA showed loops and long single polynucleotide chains due to local breakdown of the hydrogen bonds. When such molecules were renatured, they had native-like regions, alternating with protruding flower-like appearances caused by the random coiling of unmatched single strands. In the case of heated DNA, branched structures due to joining of adjacent molecules were the most prominent feature. On the other hand, DNA denatured by X-irradiation showed thinning out at places, as a prelude to complete scission of the double strand<sup>11,12</sup>.

The molecular weight of the entire DNA molecule contained within a phage is a matter of considerable importance. However, the observed length of the DNA molecule under the electron microscope is



influenced by the method of its extraction and preparation. In the present work, 184 DNA molecules from the coliphage T7 were prepared by three different methods, and their lengths measured carefully. It was found that the best estimate of the molecular weight of this DNA genome was 25. million daltons<sup>13</sup>.

DNA molecule has a dipole moment which is altered when other chemicals bind with this molecule. The dielectric properties of free DNA have been compared with those of DNA bound with the dyes acridine orange, proflavin and ethedum bromide. Such studies revealed important differences in the modes of interaction of these chemicals with DNA<sup>14,15</sup>.

### Selected Publications

1. Das Gupta N N, De M L, Bhattacharya D L & Chaudhury A K A new horizontal electron microscope, *Indian J Phys*, **22** (1948) 497-513.
2. De M L, Das Gupta N N & Guha A, Phase contrast and electron microscopic studies on the nuclear apparatus of *Escherichia coli*, *Proc R Soc*, **141 B** (1953) 199-203.
3. Dutta B N, Das Gupta N N, De M L, Guha A & Nandi S, New method for the study of intracellular parasite with the electron microscope, *Science*, **120** (1954) 428-30.
4. Chakraborty J & Das Gupta N N, Ultrastructure of the pellicle and the nucleus of *Leishmania donovani*, *Proc Fourth International Congress on Electron Microscopy, Berlin*, 1958, 510-14.
5. Chatterji K R, Das Gupta N N & De M L, Electron microscopic observations on the morphology of *Mycobacterium leprae*, *Exp Cell Res*, **18** (1959) 521-27.
6. Das Gupta N N, Bhattacharya K L, Datta Chaudhuri R D, Bose A & De P K, Uptake of radioactive phosphorus in normal breast and breast tumors, *Acts Radiol*, **45** (1956) 69-80.
7. Charkaborti K P, Bose A, Bhattacharya K L & Das Gupta N N, A study of human thyroid function by means of radioactive iodine. *J Endocr, Lond*, **10** (1954) 308-10.
8. Lala P K, Das Gupta N N & Bhattacharya S B, Life span of granulocytes in chronic leukemia, *Br J Haematol*, **8** (1962) 223-29.
9. Das Gupta N N, Misra D N, Ganguly P, Sanyal A B & Chatterjee J B, Electron microscopic and sedimentation studies on human hemoglobins A, F and E, in *Aspects of protein structure*, edited by G Ramachandran (Academic Press, London) 1963, 301-5.
10. Misra D N, Das Gupta N N, Sanyal A B & Chatterjee J B, Electron microscopic observations on human hemoglobins, *Exp Cell Res*, **34** (1964) 325-32.
11. Das Gupta N N, Sarkar M & Misra D N, Electron microscopic studies on denatured DNA from *Escherichia coli*. *J molec Biol*, **15** (1966), 619-23.
12. Bagchi B, Misra D N, Basu S & Das Gupta N N, Conformation of denatured and renatured DNA, *Biochim biophys Acta*, **182** (1969) 551-61.
13. Misra D N, Sinha R K & Das Gupta N N, Molecular weight of DNA from coliphage T7 by electron microscopy, *Virology*, **39** (1969) 183-93.
14. Goswami D N & Das Gupta N N, Dielectric behaviour of DNA-dye complex—II, *Biopolymers*, **13** (1974) 391-400.
15. Goswami D N & Das Gupta N N On the electric polarisation of DNA, *Biopolymers*, **13** (1974) 1549-56.

## Asoke G Datta

After obtaining post-doctoral training in intermediary metabolism and mechanism of enzyme action at the National Research Council, Ottawa, Canada, and in the laboratory of Prof. Efraim Racker, Datta joined the Indian Institute of Experimental Medicine and started work on various biomedical problems.

### Studies on *Vibrio cholerae*

Datta demonstrated that erythrose, a four-carbon sugar, strongly inhibited the growth of *V. cholerae* when grown in the presence of glucose or other monosaccharides in culture. The inhibition of growth was proportional to the concentration of erythrose<sup>1</sup>. Erythrose not only inhibited the organism in culture, but could also inhibit the development of experimental cholera in rabbit intestinal loop experiments; it additionally gave complete protection of development of cholera in infant rabbits when administered with live *V. cholerae* directly in the intestine<sup>2</sup>. The mechanism of inhibition was also studied by his group and they demonstrated that erythrose inhibited the growth of this organism by restricting the entry of glucose<sup>3</sup>. Although erythrose is a very strong inhibitor of the growth of *V. cholerae*, it cannot be used as a therapeutic agent, as erythrose is unable to counteract the effect of cholera toxin. However, this study indicates that

erythrose still has great potentiality as a preventive agent in endemic areas.

### Biosynthesis of Thyroxine in Extra-thyroidal Tissues

The existence of two new peroxidases in the submaxillary gland was demonstrated by Datta's group<sup>4,5</sup>; these were later purified<sup>6,7</sup>. The presence of this enzyme in human submaxillary gland was also shown and its level was found to be higher under hypothyroidic and lower under hyperthyroidic conditions. Surgical removal of thyroid glands in rats caused almost 4-fold increase in this enzyme in submaxillary glands and the increase was due to enzyme synthesis<sup>8</sup>. More recently, it has been shown by Datta's group that this enzyme not only peroxidised iodide but also catalyzed the incorporation of oxidized iodide into the tyrosine molecule to form mono- and diiodotyrosine and finally coupling of two diiodotyrosine units to produce thyroxine<sup>9</sup>. The existence of a new protein in submaxillary gland, like thyroglobulin in thyroid gland, has also been shown; it acts as a very good substrate for thyroxine formation<sup>10</sup>.

Another peroxidase from parietal cells of stomach has been discovered in Datta's laboratory<sup>11-13</sup> which shows considerable promise as a regulator of gastric HCl secretion.

---

Deputy Director, Indian Institute of Chemical Biology, Calcutta-700032; Residence : Block 1, Flat No. 5A, 59 Lake Road, Calcutta.



His latest discovery of the existence of a factor in the submaxillary gland of immature female rats which regulates the biosynthesis of sex steroids in ovary is also worth mentioning<sup>14</sup>.

### Studies on Erythropoietin

Datta's group was the first to demonstrate that kidney medullary cells are solely responsible for the formation of erythropoietin, the hormone which regulates erythropoiesis<sup>15,16</sup>.

Recently, Datta's group discovered for the first time that the effect of erythropoietin on erythropoiesis is not only through hemoglobin formation but also through enhanced lipid biogenesis of red blood cell membrane<sup>17,18</sup>. The hormone was further shown to influence the exchange of cholesterol and phospholipid between plasma and red cell membrane and also the kinetic properties of some of the red cell membrane marker enzymes<sup>19</sup>.

### Biochemical Studies on Toxaemia of Pregnancy

This disease is one of the major threats in pregnancy and the incidence of this disease is quite high in India. Very few reports have appeared on attempts to find out any biochemical changes associated with this disease. Datta's group demonstrated that in clinical cases of eclampsia and pre-eclampsia, there is a significant reduction in monoamine oxidase (MAO) with concomitant increase in plasma fibrinogen. They demonstrated in experimental animals that increase in plasma fibrinogen was triggered by the accumulation of monoamines due to inhibition of MAO. They further showed that the triggering effect was due to monoamines and not to any of their

precursors or metabolites<sup>20-22</sup>. More recently, his group has been able to demonstrate that epinephrine increases the formation of mRNA for fibrinogen through increased RNA polymerase activity and the partially purified mRNA when translated in wheat germ system *in vitro* produces fibrinogen<sup>23,24</sup>.

Besides the above-mentioned studies, which are directly related to biomedical and health problems, Datta's studies on the regulation of gluconeogenesis are worth mentioning. For his contribution in this field, he was invited twice to the Roche Institute of Molecular Biology, Nutley, NJ as a visiting scientist.

### Research and Development Studies

Datta is interested not only in basic biomedical studies but also in process development. Recently, he and his associates have developed two simple processes, one for the purification of human chorionic gonadotrophin required for the development of a diagnostic kit for early detection of pregnancy and the other for the method of isolation of wheat germ lectin, an important tool in a large number of biochemical and cell architectural studies.

His work on extrathyroidal peroxidases and biosynthesis of thyroxine has been well accepted and on the basis of this outstanding study he was awarded the B N Chopra award for 1980 and was elected a fellow of the Indian National Science Academy in 1981. He was also elected a fellow of the Indian Academy of Sciences in 1979 for his overall basic contribution in the field of biomedical research.

### Selected Publications

1. Chowdhury J R & Datta A G, Studies on the growth inhibitory effect of erythrose on *Vibrio*

- cholerae*, *Biochim biophys Acta*, **104** (1965) 296.
2. Bhattacharya P K, Chowdhury J R & Datta A G, Studies on the inhibitory effect of erythrose on the development of experimental cholera, *Br med J*, **2** (1965) 1351.
3. Nath J & Datta A G, Studies on the mechanism of inhibition of growth of *Vibrio cholerae* by erythrose, *J gen Microbiol*, **62** (1970) 17.
4. Hati R N & Datta A G, Studies on the formation of diiodotyrosine from monoiodotyrosine by a microsomal enzyme from goat submaxillary gland, *Biochim biophys Acta*, **148** (1967) 310.
5. Hati R N, Bal M & Datta A G, A potassium iodide stimulated peroxidase from goat submaxillary gland, *Biochem biophys Res Commun*, **31** (1968) 392.
6. Bal M, Sen G, Mahajani U & Datta A G, Studies on some aspects of peroxidase from submaxillary gland, *Archs Biochem Biophys*, **151** (1972) 216.
7. Mahajani U, Halder I & Datta A G, Purification and properties of an iodide peroxidase from submaxillary gland of goat, *Eur J Biochem*, **37** (1973) 541.
8. Chandra T, Das R & Datta A G, Role of thyroid gland on the peroxidase and iodinating enzymes of submaxillary gland, *Eur J Biochem*, **72** (1977) 259.
9. Guha M, Hati R N & Datta A G, *In vitro* formation of thyroid hormones from 3,5-diiodo thyronine by supernatant of submaxillary gland, *J Biosci*, **3** (1981) 239.
10. Chatterjee D K, Banerjee R K & Datta A G, Studies on peroxidase-catalyzed formation of thyroid hormones on a protein isolated from submaxillary gland, *Biochim biophys Acta*, **612** (1980) 29.
11. Banerjee R K & Datta A G, Gastric peroxidase-localization catalytic properties and possible role in extrathyroidal thyroid hormone formation, *Acta Endocr*, **96** (1981) 203.
12. Banerjee R K & Datta A G, Iodide transport and organification in extrathyroidal tissues, *Indian J Biochem Biophys*, **19** (1982) 171.
13. Banerjee R K, Bose A K, Chakraborty T K & Datta A G, Solubilisation and properties of mitochondrial peroxidase from mouse gastric mucosa, *Indian J Biochem Biophys*, **19** (1982) 324.
14. Bhattacharyya J, Banerjee P N, Datta A & Datta A G, Uterine changes on removal of submaxillary glands in rats, *Biochem biophys Res Commun*, **133** (1985) 462.
15. Chowdhury R R & Datta A G, Studies on the *in vitro* formation of erythropoietin in sheep kidney medulla and the effect of cobalt thereon, *Biochem biophys Res Commun*, **52** (1973) 1329.
16. Bandyopadhyay R & Datta A G, Further studies on the biosynthesis of erythropoietin, *Indian J Biochem Biophys*, **18** (1981) 241.
17. Chaudhuri T, Ghosal J, Ghose D K & Datta A G, Effect of erythropoietin on the [<sup>14</sup>C] acetate incorporation into the lipids of erythrocyte cell membranes, *Biochem Med*, **24** (1980) 162.
18. Ghosal J, Biswas T, Ghosh A & Datta A G, Effect of erythropoietin on the lipid composition of red blood cell membrane, *Biochem Med*, **34** (1984) 1.
19. Biswas T, Ghosal J, Ganguly C & Datta A G, Effect of erythropoietin on the interchange of cholesterol and phospholipid between erythrocyte membrane and plasma, *Biochem Med*, in press.
20. Chatterjee T, Maitra D, Chakravorty T & Datta A G, Studies on plasma fibrinogen level in pre-eclampsia and eclampsia, *Experientia*, **34** (1978) 562.
21. Sur J, Chatterjee T & Datta A G, Studies on the effect of some biogenic amines on plasma fibrinogen level of rats and rabbits, *Biochem Pharmacol*, **28** (1979) 1597.
22. Sarkar J, Roy A, Chatterjee T & Datta A G, Effect of some monoamine oxidase inhibitors and cyclic AMP on plasma fibrinogen level of rats, *Biochem Pharmacol*, **33** (1984) 539.
23. Roy A K, Bhadra R & Datta A G, The effect of epinephrine and serotonin on hepatic poly (A)<sup>+</sup> RNA synthesis, *Life Sci*, **36** (1985) 2301.
24. Roy A K, Sarkar J, Bhadra R & Datta A G, Effect of amines on fibrinogen synthesis, *Archs Biochem Biophys*, **239** (1985) 364.
25. Chatterjee T & Datta A G, Effect of glucagon administration on mice liver fructose-1,6-bisphosphatase, *Biochem biophys Res Commun*, **84** (1978) 950.
26. Banerjee R B, Bhadra R & Datta A G, Effect of glucagon on alanine 2-oxoglutarate aminotransferase, *Biochem biophys Res Commun*, **115** (1983) 506.



## G C Esh

### **Plant Foods in Relation to Varietal and Climatic Influences**

Earlier studies had shown that the nutritional value of the plant food supply, particularly of the specific food nutrients, apart from the total quantity produced, is governed by various factors, such as genetic strain and environment, including climate and soil conditions. Esh and Basu<sup>1</sup> found wide variation in the protein content of pulses, extending up to 60% in some cases, depending upon the strain used and the locality where grown. Similar variations in the vitamin content in rice and pulses were reported<sup>2</sup>. The nutritive efficiency of a high protein food<sup>3</sup> or of a food associated with high productivity<sup>4</sup> does not alter in many cases in any way. These findings could be used by the food production agencies to enrich the diet. Every plant breeding programme should take into account the nutritive value of food crops in terms of specific nutrients as well as their yield.

### **Development of Protein-rich Foods from Vegetable Sources**

Of all the dietary deficiencies, protein shortage in both qualitative and quantitative terms is the most outstanding dietary problem in India and other developing countries. Although animal

proteins, such as milk, fish, meat, eggs, etc. with their high content and well-balanced pattern of essential amino acids are usually superior to plant proteins, which are deficient in one or more essential amino acids, they are usually costly. Because of our present knowledge regarding the exact pattern of amino acid requirement for good health, it is now possible to prepare protein mixtures exclusively out of plant food materials and as such the superiority of animal proteins cannot be taken as an absolute reality any more. Pulses being the cheapest source of all the plant food materials, a series of investigations<sup>5-7</sup> were undertaken on their protein and amino acid contents, digestibility and biological value. Their digestibility and biological value are reasonably high, although there are variations from pulse to pulse. Although they are deficient in methionine/tryptophan, when supplemented with these amino acids, the nutritive value is improved significantly<sup>8</sup>. Further, their judicious mixing with or without different types of cereals, greatly improves the nutritional quality of protein. Improvement of pulse protein with vitamin B12 and other factors as well as the deterioration in a balanced protein with excess of an amino acid has been observed. How these metabolic

---

Formerly, Professor & Head, Department of Biochemistry and Vice-Dean, Faculty of Science, University of Science and Technology, Kumasi, Ghana; Residence : B/10, Government Housing Estate, P.O. Entally, Calcutta-700014.

changes occur is yet to be studied exhaustively<sup>9</sup>.

### **Alteration of the Amino Acid Pattern of Plant Foods**

Vegetable protein is most readily available in the cereal grains, and the leguminous seeds, but usually the former lack in lysine and the latter in methionine/tryptophan. Through genetic improvement of the food crops, however, the amino acid composition can be satisfactorily altered. New hybrid corns with high lysine content have already been developed. Similar improvements can be made in wheat and rice<sup>10</sup>.

### **Extraction of Protein from Leaf**

Young vigorously growing leaves often contain about 40% protein and little fibre. Those leafy crops which are not directly consumed by man or animal can be fruitfully used for the production of protein by a suitable technique which may well supplement poor diets<sup>10a</sup>.

### **Alteration of the Pattern of Food Production**

The main cereal crops like rice, wheat, jowar, bajra and pulses cover 80-90% of the total cultivable area for food production. Depending upon the food consumption habits of our people and considering the various aspects of production, it is high time that we revise the pattern of production. This is more true regarding the production of subsidiary foods like roots and fruits. Data presented indicate clearly that in crops like potato, sweet potato, banana and papaya not only the yield per acre is much greater than that of rice and wheat, the return in terms of protein and total calorie is also much higher<sup>10</sup>.

### **Studies on Vitamin A**

(a) *Influence of soy-lecithin on the absorption and utilization of vitamin A* : Young pregnant dairy animals were fed vitamin A with or without lecithin, one month prior to parturition. At parturition, higher plasma vitamin A levels were observed in cows fed lecithin-supplemented vitamin A, indicating that lecithin enhances the absorption of vitamin A. Higher colostrum vitamin A at the first milking indicates increased transmission of the vitamin by lecithin. Higher blood plasma vitamin A level in the newborn calves as well as higher total liver storage vitamin A indicate that lecithin enhances the absorption and utilization of vitamin A<sup>11</sup>. Similar enhanced capacity of lecithin was observed when rats were taken as the experimental animals<sup>12</sup>.

(b) *Inter-dependence of vitamins A, B-complex, vitamin C and protein in metabolism* : A series of investigations were undertaken to study the possible inter-relationship of dietary protein and vitamin A in metabolism, with special reference to vegetable proteins. During amino acid imbalance induced by feeding vegetable proteins, higher intake of vitamin A not only enhances the body weight gain but also increases the activity of certain liver enzymes and nitrogen retention significantly<sup>13</sup>. Higher nitrogen excretion, lowering of plasma protein and decreased rate of body nitrogen deposition during growth associated with vitamin deficiency states, suggest that absence of vitamin A results in failure of tissue growth in growing animals; thus, normal protein catabolism is enhanced, resulting in the breakdown of body tissues<sup>14</sup>. The role of vitamin A in the synthesis of body proteins has been indicated<sup>15</sup>. That the imbalance



brought about by maintaining the animals of low protein diets demands more vitamin A to attain nitrogen equilibrium at the minimum dietary nitrogen level suggests a protein sparing effect of vitamin A. Further, the impairment of storage or aggravation in the depletion of liver reserve of vitamin A during amino acid imbalance is possibly due to its rapid withdrawal from the liver under such conditions and is perhaps related to its utilization in the system.

The necessity for optimum supply of B vitamins for optimum mobilization of dietary protein, particularly at higher levels of protein intake and imbalanced protein supply, has been indicated<sup>16</sup>. Higher weight gain, higher nitrogen efficiency ratio and concentration of certain enzymes in the liver, such as xanthine oxidase, cholinesterase, succinate dehydrogenase and alanine amino transferase were observed with increasing supplementation of B vitamins<sup>17</sup>.

The series of investigations with guineapigs indicated a possible interdependence of dietary protein and vitamin C in metabolism<sup>18</sup>. The data indicate that the vitamin is not utilized adequately under the condition of feeding an imbalanced protein. Higher tissue storage of the vitamin along with optimum weight gain with high intakes of the vitamin indicate highly efficient and economic utilization of both the dietary protein and the vitamin.

The interdependence of vitamin C and protein in metabolism under high level of vitamin A feeding has also been followed<sup>19</sup>.

### **Influence of Carbohydrates on Protein Metabolism**

Studies on the influence of different

types of carbohydrates on protein metabolism in rats indicated that the dietary protein modifies the responses of enzyme synthesis to changes in dietary carbohydrates<sup>20</sup>. Complex carbohydrates, such as starches and dextrin, showed beneficial effect on growth with casein and groundnut protein.

### **Metabolic Relationship between Dietary Protein and Iron**

The requirement of dietary protein for optimum iron storage has been found to be much lower than that required for optimum growth<sup>21</sup>. But a grossly imbalanced protein like gelatine when fed at low levels to rats reduces the normal storage of iron considerably. Protein has little influence on the utilization of stored iron, although it is necessary for efficient absorption and storage of iron and for increasing haemoglobin concentration, showing the beneficial effect of protein in absorption. The data indicate that the protein degradation products are required in small amounts for efficient storage. The findings suggest that peptides or amino acids act as vehicles for the transport of iron through the gastro-intestinal mucosa.

### **Studies on the Presence of Carcinogenic Hydrocarbons**

Some polycyclic hydrocarbons, such as benzo(a)pyrene, dibenzpyrene and benzanthrane, have been reported to be carcinogenic at least in certain laboratory animals. In view of the fact that the formation and the quantity of such hydrocarbons vary widely, depending upon the nature and extent of smoking the fish, four samples of Ghanaian smoked fish were tested<sup>22</sup>. Significant quantities of benzo(a)pyrene and 3:4:9-dibenzpyrene have been observed in the samples

studied. More detailed studies on the subject need to be carried out.

### Studies on Digestive Enzymes

The digestive enzymes, such as pancreatin, papain, trypsin and amylase (diastase) can be easily prepared from natural sources. The method for their isolation, purification and estimation of stability have been discussed. Their large-scale production should be encouraged<sup>23,24</sup>.

### Selected Publications

1. Esh G C, De T S & Basu U P, Influence of genetic strain and environment on the protein content of pulses, *Science*, **129** (1959) 148.
2. Esh G C & De T S, Association with thiamine and nicotinic acid content of pulses, *J Proc Instn Chem*, **31** (1960) 241.
3. Esh G C, De T S & Basu U P, Nutritive value of the proteins of Bengal gram of high and low protein content, *Br J Nutr*, **14** (1960) 425.
4. Esh G C & Basu U P, The relation between the yield and nutritive value of wheat, *Indian J agric Sci*, **22** (1952) 275.
5. Esh G C & Som J M, Nutritive value of pulses, *Indian J Physiol all Sci*, **6** (1952) 61.
6. Esh G C & Som J M, Availability of methionine, cystine and tryptophan in pulses, *Indian J Physiol all Sci*, **7** (1953) 158.
7. Esh G C, Pulses as food protein, *J Proc Instn Chem India*, **30** (1958) 21.
8. Esh G C & Som J M, Studies on the nutritive value of plant proteins, *Proc natn Inst Sci India*, **21B** (1955) 68.
9. Esh G C & De T S, Variability in the metabolic pattern of dietary proteins, *Bull natn Inst Sci India*, **19** (1962) 183.
10. Esh G C, How to meet challenge of protein food shortage, *Proc Symp on Science and India's Food Problem*, 1967, 480.
- 10a. Esh G C, Leaf proteins as our food, *J Sci Club*, **8** (1955) 127.
11. Esh G C, Sutton T S, Hibbs John & Krause W E, The effects of soy-phosphatides on the absorption and utilization of vitamin A, *J Dairy Sci*, **31** (1948) 461.
12. Esh G C & Sutton T S, The effect of soy-lecithin on the absorption and utilization of vitamin A, *J Nutr*, **36** (1948) 391.
13. Esh G C & Bhattacharya R K, Protective influence of vitamin A during amino acid imbalance, *Ann Biochem exp Med*, **22** (1962) 147.
14. Esh G C & Bhattacharya R K, Influence of vitamin A on the metabolism of dietary nitrogen, *Ann Biochem exp Med*, **21** (1961) 215.
15. Esh G C & Bhattacharya R K, Influence of vitamin A during protein depletion and regeneration, *Ann Biochem exp Med*, **23** (1963) 467.
16. Esh G C, Som J M & Kar M K, Influence of the level of certain B-vitamins on dietary proteins, *Indian J Biochem*, **3** (1965) 267.
17. Esh G C, Som J M & Kar M K, Influence of the levels of B-vitamins during protein regeneration, *Indian J Biochem*, **4** (1967) 130.
18. Esh G C, Bhattacharya S P & Basu U P, Influence of vitamin C during protein regeneration in guineapigs, *Metabolism* **15** (1965) 1039.
19. Esh G C & Bhattacharya S P, Influence of vitamin C on protein metabolism, *Indian J Biochem*, **3** (1966) 196; Relative influence of vitamin A and ascorbic acid on protein depletion and regeneration, *Indian J Biochem*, **4** (1967) 114.
20. Esh G C & Bandopadhyaya P, Metabolic changes as influenced by types of carbohydrates and protein, *Indian J Biochem*, **5** (1968) 173; Influence of types of carbohydrates on the progress of protein depletion and regeneration, *Indian J Biochem*, **5** (1968) 178.
21. Esh G C & Bhattacharya R K, Quality of protein and iron storage, *Indian J Biochem*, **1** (1964) 169; Metabolic relationship between dietary protein and iron, *Indian J Biochem*, **1** (1964) 49.
22. Quayson C K E, Graham C J & Esh G C, Studies on the presence of carcinogenic hydrocarbons in smoked fish, *Ghana med J*, **13** (1974) 188.
23. Esh G C, Kar M K & Misra A L, Studies on diastase from *Aspergillus oryza*, *J Proc Instn Chem India*, **43** (1971) 221.
24. Esh G C, Enzymes in pharmaceutical preparations, *Proc Seminar on the Development of Indian Technical Knowledge* (All India Manufacturers' Organization) 1966, 15.



# K Ganapathi

## Chemistry of Pinene Derivatives

Pinic acid, a degradation product of  $\alpha$ -pinene, was presumed to have the *cis*-configuration. Against this, it was proved to have the *trans*-configuration by synthesizing it from *cis*-norpinic acid and making a comparison<sup>1</sup>. Starting from *cis*-norpinic acid, pinononic acid, homopinic acid and diketonorpinane were synthesized; efforts to synthesize pinocamphone proved infructuous. The stereochemistry of the bicyclic pinane system was studied to elucidate the various isomeric compounds previously reported, and also to explain the Meerwin-Wagner transformation and the types of monocyclic compounds produced by fission of the bicyclic ring<sup>2</sup>. On the basis of the study of the relative abundance of various terpenes in the essential oils in which  $\alpha$ -pinene predominates, a scheme of biogenesis of the mono and bicyclic terpenes was proposed, postulating linalool as having the suitable structure to serve as the basic terpene.

## Trans-Decalin Derivatives and Stereochemical Projection Representation

2:3-*Trans*-Diketodecalin was synthesized from *trans*- $\beta$ -decalone by selenium dioxide oxidation; its structure was proved and the report in the literature was found to be in error. By various methods, three

2:3-dihydroxy *trans*-decalins were synthesized and their reactions established. Configurations were assigned to them based on the old forms of *cis*- and *trans*-decalins; the projection formulae were used for the first time in this; the projections obtained are now known as Herman projections<sup>3</sup>.

## Studies in Sulpha Drugs

Following the sensational discovery of prontosil by Gerhardt Domag, extensive studies were made on this group of antibacterial drugs<sup>4</sup>. About 400 sulphanilamide derivatives of numerous types were synthesized and a selected number was tested for protection against *Streptococcal*, *Pneumococcal* and *P. pestis* (and some other) infections in mice. A dozen compounds with outstanding protective effects were discovered. For the first time, 2-sulphanilamidothiazole (now known as sulfathiazole), discovered independently, was established to give protection against plague infections in mice; it was far superior to sulphapyridine in this respect. Enough of this drug was produced and tried in human plague infections in the field in comprehensive clinical trials, and the therapeutic effect was confirmed<sup>5</sup>. Thus, the dreaded plague infections could be cured by the sulfa drugs. Sulphathiazole was also formulated

---

Formerly, Director, Regional Research Laboratory, Jammu; Residence : B-23, Jayanti Apartments, XIII Cross Road, Malleswaram, Bangalore-560003.

into a paste to be applied topically to give cures in wounds and burns. The other drugs discovered were : sulphaguanidine, sulphamethazine and sulphadimidine. A method for producing sulphathiazole was worked out wherein acetsulphanilyl chloride (ASC) could be used in wet condition and the condensation with 2-amino thiazole was effected in aqueous media, with great practical advantages. This process was patented<sup>6</sup> and the drug manufactured in quantity.

### Chemotherapy of Malaria

In search for possible antimalarials which will have effect on the exoerythrocytic forms of the malaria parasite, about 200 compounds of quinoline, thiazole, guanidine, biguanides, uracil, thriouracil and pteridines were synthesized and studied in *Plasmodium berghii* infections in mice.

A comprehensive review of the subject covering all aspects, such as the cycle of the malaria parasite between man and the mosquito, the various stages of the parasite and their implications in therapeutic control, as the sporozoites, exoerythrocytic forms, erythrocytic forms, gametocytes, the various methods of testing of the drugs, was published<sup>7</sup>.

### Chemistry of the Thiazoles

This work was undertaken to explore different methods of production of sulphathiazole and also the thiazole moiety of the vitamin thiamine. Methods for the production of 2-amino, 4-amino and 5-aminothiazoles as well as to produce sulphanilamide derivatives from these were developed. A simplified and efficient method for preparing 2-chlorothiazole and 2-halogenothiazoles was worked out and patented<sup>8</sup>. The parent compound, thiazole,

a curiosity so far, was prepared in quantity and its reactions studied. 2-Amino-5-nitrothiazole was synthesized which was discovered by others to have good antiprotozoal activity. The orientation in the thiazole nucleus by nitration and bromination of various thiazole derivatives was studied; marshalling all the evidence so far, the fine structure of the thiazole ring was clarified to explain the various types of reactions the thiazole compounds undergo. A critical review of the chemistry of the thiazoles prepared was not published due to the appearance of a book, but was incorporated in the thesis of a colleague.

### Laboratory Scale Penicillin Production

Following the publication of the paper by H Florey and E B Chain on penicillin, we undertook laboratory production of penicillin in surface cultures using thousands of glass bottles and various types of media, containing bran and 'gud'. The penicillin produced was extracted as per the methods described and the final freeze-dried products were obtained. We came across the enigmatic penicillin X, but missed it because we relied on the information contained in literature!

### Mechanism of Biosynthesis of Penicillin

Marshalling all the apparently disconnected facts available, a mechanism of biosynthesis of penicillin by the mould *Penicillium chrysogenum* was suggested as a diversion from protein synthesis from the available amino acids, when the growth phase of the mould had stopped<sup>9</sup>. The disintegrated mycelium did not produce penicillin in shaken cultures; the integrity of the mycelium was essential. An experimental system was established in



which the washed cells were suspended in a suitable synthetic medium and the cultures shaken. The materials to be investigated were added to the medium and the penicillin production measured. Penicillin synthesis was stimulated by a variety of carbohydrates, inositol, glycerol, fatty acids, fatty oils, and many amino acids, while the Krebs's cycle intermediates did not cause any stimulation. The biosynthesis was inhibited by dinitrophenol, cyanide and arsenite, implicating the electron transport system in the biosynthesis. It was postulated that the compounds that caused stimulation were serving as sources of continuous production of ATP molecules required to form the peptide bonds, and the removal of two hydrogen atoms, dehydrogenation, was postulated to be mediated by the cytochromes in the respiratory chain.

#### **Cellular Constituents of *P. chrysogenum***

The constituents of the mycelium as obtained in the penicillin factory were analyzed. Chitin was detected by the presence of acetylaminoglucose in the hydrolysate. The lipid fraction contained the usual fatty acids. Mannitol (2%) and tiglic acid were isolated. The sterol content was less than 0.1% with probably ergosterol predominating.

#### **Carbohydrate Metabolism of *P. chrysogenum***

Studied in shaken cultures, *P. chrysogenum* utilizes a variety of carbohydrates (18 in number studied) and converts all of them into glucose, which seems to polymerize into glucan. A difference was noted between the 'seed mycelium' which does not produce penicillin and the 'mature mycelium' which does so. The mature mycelium contained

10-20% of free reducing sugars in the cold water extract, called the 'free sugar pool'; this was depleted in the seed mycelium. In the mature mycelium, irrespective of the sugar used for growth promotion, the free sugar pool contained mostly glucose, with four more sugars detected by paper chromatography; free ribose was present. The seed mycelium contains in the aqueous extract fructose and ribose with a small amount of glucose. Hydrolysate of the extract yielded galactose. Thus, it appeared that numerous pathways involving the carbohydrates function in the mould, explaining the vast adaptabilities of the moulds to varying environmental conditions.

In the course of this work, techniques were developed to work with 25-50 mg of the mycelia, and identify the various pentoses and hexoses by paper chromatography and resorcinol-sulphuric acid reaction which give characteristic spectra for each sugar<sup>10</sup>. Taking advantage of the differences found between ribose and deoxyribose, a method for differential assays of DNA and RNA was also worked out<sup>11</sup>.

#### **Biochemistry of *Pasteurella pestis*, *Vibrio cholera* and *Bordetella pertussis***

A comparative study of the biochemistries of the pathogenic bacteria was undertaken by using <sup>14</sup>C labelled sugars, amino acids, purines and pyrimidines, and incorporation into the cellular constituents. Shaken cultures were used; the bacterial mass obtained was fractionated into ether soluble, water soluble, and hydrolyzed fractions. Presence of radioactivity was detected by the usual counting methods. Sialic acid was detected in the cells of *P. pestis* and in

many gram-positive and gram-negative bacteria<sup>12</sup>.

### Citric Acid Production by Submerged Fermentation Using *Aspergillus niger*

A suitable strain of *A. niger* was selected, which could convert in shaken cultures and in 1 gal stirred fermentor, sucrose into citric acid in high yields. The system was not sensitive to iron. Use of molasses in place of sucrose gave erratic results because of the varying compositions of molasses from different sources<sup>13</sup>.

### Pilot Plant and Industrial Work

A process for the production of sulphathiazole was worked out, as indicated earlier and by this, sulphathiazole was produced in tonnage lots.

A process for the production of the antimalarial paludrine was worked out starting from basic raw materials. This was done in 50 kg batches and in good yields.

Vitamin A concentrate was produced from the locally available shark liver oil, from the unsaponified portion by solvent extraction. This extract was incorporated into vitamin tablets.

Diosgenin was produced from *Dioscorea deltoidea*. The roots were pulverized, acid hydrolysed, the plant material washed and dried, and extracted with solvent oil; diosgenin obtained in good yield and of fairly good purity could be utilized for the production of steroid hormones.

In the production of penicillin in the plant, a number of modifications in the medium were introduced, like the use of peanut meal partly replacing corn steep extracts, periodic addition of sucrose in place of imported lactose. Penicillin V, the 'oral penicillin', was produced by using phenoxyacetic acid as the precursor in place of phenylacetic acid.

### Selected Publications

1. *Ber dt chem Ges*, **70** (1973) 1505.
2. *J Indian Inst Sci*, **22A** (1939) 155-64.
3. *Ber dt chem Ges*, **72** (1939) 1381-86.
4. *J scient ind Res*, **10A** (1951) 481.
5. *Indian med Gaz*, **76** (1941) 29.
6. *Proc Indian Acad Sci*, **18A** (1943) 360; *Indian Pat* 29093.
7. *Indian J Pharm* (1947) 1948.
8. *Proc Indian Acad Sci*, **22A** (1945) 362-67; **38A** (1953) 45-57.
9. *Experientia*, **13** (1957) 172-75.
10. *J scient ind Res*, **19C** (1960) 207-22.
11. *Indian J Biochem*, **1** (1964) 26-30.
12. *Nature, Lond*, **183** (1959) 758.
13. *Res & Ind, New Delhi*, **16** (1971) 99.



## J Ganguly

The important contributions of Ganguly are summarized below.

### (I) Absorption and Metabolism of Carotenoids and Vitamin A

Ganguly has worked in these areas continuously for nearly 40 years and has made several far-reaching observations of fundamental importance.

(1) *Carotenoids* : It used to be widely accepted that the dietary carotene is converted into vitamin A in the liver. But during his PhD work, Ganguly had demonstrated that when carotene is fed to rats, vitamin A appears in the intestine rapidly long before it can be found in the liver, which obviously proved that the conversion takes place in the intestine and not in the liver.

During this work, numerous attempts to demonstrate *in vitro* conversion with tissue preparations were uniformly unsuccessful<sup>1</sup>. Eventually, work from other laboratories revealed that the *in vitro* conversion is very inefficient and that dioxygenase enzyme systems isolated from animal tissues, when incubated with carotene, can give retinal. Isolation and identification of retinal from such systems had led to the universal belief that the dioxygenase specifically attacks the central double bond of carotene, giving two molecules of retinal. On the basis of well-established biological carotene/vitamin A

relationship, Ganguly has recently challenged the idea of specific central cleavage and has put forward a novel mechanism involving random cleavage of the carotene molecule<sup>2</sup>. This mechanism is being accepted rapidly.

Ganguly *et al.*<sup>3</sup> had isolated a new provitamin A from sea urchin eggs and had demonstrated that it is 4-oxo- $\beta$ -carotene. It was also shown that it is 50% as active as  $\beta$ -carotene in promoting the growth of vitamin A-deficient rats.

(2) *Vitamin A* : Ganguly had earlier shown that vitamin A is absorbed through the lymphatic system as its ester<sup>4</sup>. His later sustained work revealed that the dietary retinyl esters are hydrolyzed in the lumen of the small intestine while in the fat phase, after which the retinol appears in the micellar phase. From such micellar solution, the retinol is absorbed into the mucosal cell, where it is re-esterified mainly with palmitic acid. It was most remarkable that even when retinol was given to rats in diets containing a mixture of different fatty acids, mostly retinyl palmitate was found in the mucosal cells<sup>5,6</sup>.

Long before the concept of transport proteins and receptor proteins for steroids had emerged, Ganguly demonstrated that retinol is transported in blood by a specific carrier protein and is stored in the liver in association with a binding protein<sup>4,7,8</sup>. In

---

Emeritus Scientist (ICMR), Department of Biochemistry, Indian Institute of Science, Bangalore-560012;  
Residence : 281, Raj Mahal Vilas Extension, Bangalore-560012.

recent years, such proteins have been isolated and there has been extensive work on these proteins<sup>9</sup>.

(3) *Retinoic acid*. This compound was synthesized by Arens and van Dorp in 1946, when they had concluded that even though it supports the growth of vitamin A-deficient rats, it is not absorbed and stored in the animal body. But Ganguly<sup>6</sup> demonstrated that it is absorbed rapidly through the portal route and is quickly excreted through the bile of rats. It was also shown that it is markedly more active than retinol in promoting the growth of vitamin A-deficient rats, soon after it is given to the rats<sup>10</sup>. Mahadevan *et al.*<sup>11</sup> had found that rat liver contains enzymes which can oxidize retinol to retinal and retinal to retinoic acid, which indicated that the acid can be a natural metabolite of retinol.

(4) *Systemic mode of action of vitamin A*: The manner in which vitamin A functions outside the visual system has been a highly challenging problem. After the co-enzymic role of many of the water-soluble vitamins was established during the 1960's Ganguly had actively investigated the question of similar co-enzymic functions of vitamin A. But all attempts in these directions proved fruitless. Eventually, after critically analyzing the existing observations regarding the effects of vitamin A deficiency in animal tissues, Ganguly pointed out that it is required for normal division and differentiation of cells in general, and of epithelial cells in particular. By using systems like regenerating rat liver (following partial hepatectomy), oestrogen-induced development of chick oviduct and spermatogenesis in rat testes, he was able to provide sufficient evidence in support of his hypothesis. He has recently reviewed

the entire field and has drawn attention to the similarities in the biogenesis, mode of transport and ultimate action at the chromatin level of retinol and steroid hormones. Such similarities have led him to suggest that retinol should be called a co-steroid hormone<sup>9</sup>.

## (II) Cholesterol

As early as 1962, Murthy and Ganguly<sup>12</sup> had shown that the bile salt (sodium taurocholate) acts as a cofactor for cholesterol esterase and that synthesis and hydrolysis of cholesterol esters need not be reversible processes carried out by the same enzyme. It was also shown that feeding of a diet containing 1% cholesterol markedly induces the cholesterol-esterifying activity of rat-intestinal mucosa. More recently it was demonstrated by Paul *et al.*<sup>13</sup> that following feeding of triglycerides and phospholipids containing high proportions of unsaturated fatty acids, excretion of cholesterol through the bile of rats is increased markedly, which led them to suggest that the pronounced hypocholesterolemic effects of unsaturated lipids could be due to such increased elimination of cholesterol through the bile and ultimately through the faeces.

## (III) Fatty Acid Synthesis

Contrary to the well-accepted views prevailing at that time that the synthesis of fatty acids takes place through a simple reversal of the steps involved in  $\beta$ -oxidation, Wakil and Ganguly<sup>14</sup> had demonstrated that the synthesis takes place via carboxylation of acetyl-CoA to malonyl-CoA. In this mechanism, acetyl-CoA is first carboxylated by a biotin-containing enzyme to malonyl-CoA, which, in turn, condenses with another molecule of acetyl-CoA with simultaneous



decarboxylation. Ganguly<sup>15</sup> then showed that carboxylation is an important regulatory step in fatty acid synthesis. These observations of Ganguly proved to be rather revolutionary and paved the way for extensive work on the regulation of metabolism of higher fatty acids.

### Selected Publications

1. Ganguly J & Murthy S K, Biogenesis of carotenes and vitamin A in "The Vitamins", Vol. 1, edited by W H Sebrell & R S Harris (Academic Press Inc., New York) 1967, 125-53.
2. Ganguly J & Sastry P S, Mechanism of conversion of carotene to vitamin A—Central cleavage versus random cleavage, *Wld Rev Nutr Diet (Karger)*, **45** (1985) 198-220.
3. Ganguly J, Krinsky N I & Pinckard, Isolation and nature of echinene, a provitamin A, *Archs Biochem Biophys*, **60** (1956) 345-151.
4. Ganguly J, Absorption, transport and storage of vitamin A, in *Vitamins and Hormones*, Vol 18 (Academic Press, New York), 1960, 384-402.
5. Mahadevan S & Ganguly J, Further studies on absorption of vitamin A, *Biochem J*, **81** (1961) 53-58.
6. Ganguly J, Absorption of vitamin A, *Am J clin Nutr*, **22** (1969) 923-33.
7. Ganguly J, Krinsky N I, Mehl J W & Deuel H J (Jr), Studies on distribution of vitamin A as ester and alcohol and carotenoids in the plasma proteins of several species, *Archs Biochem Biophys*, **38** (1952) 275-82.
8. Ganguly J & Krinsky N I, Absence of relationship between vitamin A alcohol levels in plasma and liver of rats *Biochem J*, **54** (1953) 177-181.
9. Ganguly J, Rao M R S, Murthy S K & Sarada K, Systemic mode of action of vitamin A in *Vitamins and Hormones*, Vol 38 (Academic Press, New York) 1980, 1-54.
10. Malathi P, Subba Rao K, Seshadri Sastry P & Ganguly J, Studies on metabolism of vitamin A. I. Biological activity of vitamin A acid in rats, *Biochem J*, **87** (1963) 305-11.
11. Mahadevan S, Murthy S K & Ganguly J, Enzymic oxidation of vitamin A aldehyde to vitamin A acid by rat liver, *Biochem J*, **85** (1962) 326-31.
12. Murthy S K & Ganguly J, Studies of cholesterol esterases of the small intestine and pancreas of rats, *Biochem J*, **83** (1962) 460-69.
13. Paul R, Ramesha C S & Ganguly J, On the mechanism of hypocholesterolemic effects of polyunsaturated lipids, *Adv Lipid Res*, Vol 17 (Academic Press, New York) 1980, 155-71.
14. Wakil S J & Ganguly J, On the mechanism of fatty acid synthesis, *J Am chem Soc*, **81** (1959) 2597.
15. Ganguly J, Studies on the mechanism of fatty acid synthesis. VII. Biosynthesis of fatty acids from malonyl-CoA, *Biochim biophys Acta*, **40** (1960) 110-118.

## V Jagannathan

### (1) Biochemistry

The main work of Jagannathan in biochemistry was in the field of enzymes. Phosphorylated phosphoglucomutase was shown for the first time to be an intermediate during the catalytic activity of this enzyme. Pyruvic oxidase from pigeon breast muscle was isolated as a high molecular weight multi-enzyme complex. It was shown to produce "active acetate" as an intermediate, which could acetylate sulfanilamide. Pyruvate oxidase was separated from  $\alpha$ -ketoglutarate oxidase, which was also solubilized. Other enzymes which were obtained in soluble form and purified for the first time were hydrogenase from *desulfovibrio desulfuricans* and hexokinase, acetyl cholinesterase and NADP glycohydrolase from ox brain. Several new enzymes were discovered, which include a TPN specific glycerol dehydrogenase and a calcium requiring phytase specific for inositol phosphates. The glycolytic enzymes of *Aspergillus niger* were characterized and their variation during citric acid production was studied. The aldolase of this organism and other fungi was shown for the first time to be a metal requiring enzyme. A large number of specific plant proteinase inhibitors were found to be present in *Vigna* and the subtilisin inhibitors and papain inhibitors were isolated in pure form. It was also shown that papain

inhibitors were present in all plants which were examined. A high cellulase producing *Penicillium* was isolated and several of the endo- and exo-cellulases and  $\beta$ -glucosidases were obtained in pure form and their properties studied.

### (2) Plant Tissue Culture

The application of plant tissue culture to agriculture and forestry was established for research on clonal propagation, virus elimination, mutation and embryo culture. The multiplication of elite mature forest trees was achieved for the first time by the development of methods for the clonal propagation of 100 year old teak and 30 year old eucalyptus. Other species of importance to India for which clonal propagation methods were developed were tamarind, pomegranate, cardamom, ginger and turmeric. By the use of meristem culture, virus-free sugarcane and banana plants were obtained; field tests on the former gave significantly higher yields compared to the virus infected plants. By the use of embryo culture, virus resistant papaya hybrids were obtained. Variants or mutants of higher yielding wheat and high curcumin containing turmeric were obtained by tissue culture.

### (3) Proposed Lines of Work

A centre for research in the application of biotechnology to agriculture is being



established at the Tata Energy Research Institute, a non-commercial organization in Delhi. The main objective is research in plant biochemistry and physiology and DNA recombinant technology and its application to agriculture, horticulture and forestry.

### Selected Publications

1. Jagannathan V & Luck J M, Phosphoglucomutase, I—Purification and properties, *J biol Chem*, **179** (1949) 56.
2. Jagannathan V & Luck J M, Phosphoglucomutase, II—Mechanism of action, *J biol Chem*, **179** (1949) 569.
3. Jagannathan V & Schweet R S, Pyruvic oxidase of pigeon breast muscle—I. Purification and properties of the enzyme, *J biol Chem*, **196** (1952) 551.
4. Jagannathan V & Schweet R S, Pyruvic oxidase of pigeon breast muscle—II. Physico-chemical studies, *J biol Chem*, **196** (1952) 563.
5. Jagannathan V & Kartar Singh, Glycolytic enzymes of *Aspergillus niger*, *Enzymologia*, **16** (1953) 150.
6. Sadana J C & Jagannathan V, Purification of hydrogenase from *Desulfovibrio desulfuricans*, *Biochim biophys Acta*, **14** (1954) 287.
7. Sadana J C & Jagannathan V, Purification and properties of the hydrogenase of *D. desulfuricans*, *Biochim biophys Acta*, **19** (1956) 440.
8. Jagannathan V, Kartar Singh & Damodaran M, Purification and properties of aldolase from *A. niger*, *Biochem J*, **64** (1956) 477.
9. Baliga B S, Bhatnagar C M & Jagannathan V, TPN-specific glycerol dehydrogenase from *A. niger*, *Biochim biophys Acta*, **58** (1962) 384.
10. Joshi Mira & Jagannathan V, Properties and kinetics of purified brain hexokinase, *Archs Biochem Biophys*, **125** (1968) 460.
11. Kaplay S S & Jagannathan V, Purification and properties of ox brain acetylcholinesterase, *Archs Biochem Biophys*, **138** (1970) 48.
12. Hendre R R, Mascarenhas A F, Nadgir A L, Pathak M & Jagannathan V, Growth of mosaic virus-free sugarcane plants from apical meristems, *Indian Phytopath*, **28**(2) (1975) 175-78.
13. Gupta P K, Nadgir A L, Mascarenhas A F & Jagannathan V, Clonal multiplication of *Tectona grandis* Linn (teak) by tissue culture, *Pl Sci Lett*, **17** (1980) 259.
14. Gupta P K, Mascarenhas A F & Jagannathan V, Clonal propagation of mature trees of *Eucalyptus citriodora* Hook by tissue culture, *Pl Sci Lett*, **20** (1981) 195-201.
15. Rele Meenakshi V, Vartak H G & Jagannathan V, Proteinase inhibitors from *Vigna unguiculata* subsp *cylindrica*, 1. Occurrence of thiol proteinase inhibitors in plants and purification from *Vigna unguiculata* subsp *cylindrica*, *Archs Biochem Biophys*, **203**(2) (1980).

## C R Krishna Murti

### (1) Initiation

I had no inkling at all during my high school, undergraduate and graduate education (1928-1942) that I was going to be tied up with biochemical research for the rest of my professional career. My graduate education gave me a rudimentary training to seek employment in the chemical industry. It was, however, my first job in a pharmaceutical company in Calcutta (1942-1943) that exposed me for the first time to one aspect of applied biochemistry. An offer of a Rs 40 monthly stipend by the Department of Industries, Madras Presidency, to do research in the Department of Biochemistry, Indian Institute of Science, Bangalore, then and now the Mecca of biochemical research in India, led to my giving up a Rs 90 monthly paid job in industry and plunging into research in August 1943.

### (2) Deeksha

The late Prof. V Subrahmanyam, the then head of the Department of Biochemistry, was already guiding a number of projects for defence needs. After suggesting that I should work on pectins, he handed me over to the late Prof. K V Giri. Mercifully, I was left all alone to find an anchorage in an uncharted sea. My exposure to carbohydrate chemistry was so poor that I wasted two years to learn that a fraction laboriously isolated by us from tamarind

seed kernel was not after all a pectin. We had, however, better luck with immature tamarind and unripe papaya and guava. It was gratifying to learn later that the Central Food Technological Research Institute, Mysore, used the latter finding to develop commercial processes for pectin<sup>1</sup>.

From pectin to ficin, the milk clotting enzyme of the fig latex, was promotion to enzymology. After learning the first lesson of enzyme assay from no less a person than Dr V Jagannathan, I succeeded in purifying the enzyme by using calcium phosphate and alumina gels prepared and aged in our laboratory. Before the advent of ion exchange resins and column chromatography, separation of the milk clotting component from the proteolytic component achieved by us must have been due to a rare piece of luck<sup>2</sup>. Years later, along with Bruce Stone, I had the opportunity of separating for the first time  $\beta$ -glucosidase from the glucanases acting on higher oligomers of cellobiose using Dowex-2 anionic resins<sup>3</sup>. The above separation methods have found their place in Long's *Handbook of Biochemistry*.

In the course of about five years of postgraduate work in Bangalore, my kit contained an assortment of tools and untested skills to conduct nutritional investigations on rats, inmates of orphanages and adult human volunteers.

---

Chairman, Scientific Commission on Bhopal Gas Leakage, Cabinet Secretariat, A228, Sardar Patel Bhawan, New Delhi-110001.



The disadvantages of having such a wide base of initial training were soon brought home to me when I faced unsuccessfully two interviews in succession. In the first interview, which was for Assistant Research Officer in Nutrition at Coonoor, the expert told me that my training was in enzymology. In the second interview, which was for a Lecturer in Enzymology, the same expert bowled me out declaring that my expertise was in nutrition.

### (3) Career

The demonstration of the inhibition of the clotting activity of ficin and papain by allicin, streptomycin and penicillin was the beginning of a decade's obsession with the mechanism of action of antibacterial agents at the enzyme level. The late Sir Edward Mellanby, the first Director of the Central Drug Research Institute (CDRI), Lucknow, was reluctant to believe that enzyme inhibition by antibacterial agents and reversal by sulphhydryl agents could have been demonstrated by an upstart biochemist in India and not by an accredited authority like Dr T S Work of England. At any rate, once convinced of the veracity of the finding, he gave me the green signal to go all out. That streptomycin could be exerting its primary action on the energetics of the cell by disrupting the plasma membrane of sensitive organism was an exciting lead those days<sup>4</sup>. Later, we could also present a plausible mechanism of action for isonicotinic acid hydrazide<sup>5</sup>.

### (4) Pathogenic Microbes and Biochemical Parasitology

The delineation of the pathways of intermediary metabolism of amino acids and sugars in pathogenic vibrios gave us the much-needed training in diverse

methodologies of handling microbes. The work on the enzymology of cholera<sup>6</sup> was extensively quoted in Pollitzer's monograph on *V. cholerae* for WHO and was recognized in my subsequent election to the Fellowship of the Indian National Science Academy. However, in this work, spread over two decades, we had two failures: (i) our missing to recognize the role of cyclic AMP phosphodiesterase, and (ii) our inability to take advantage of the many new auxotrophs of *V. cholerae*.

With the shift in emphasis from infectious diseases to parasitic diseases in the R&D programme of CDRI, we moved on to the biochemistry of amoebic and helminth parasites. The development of a model using a free-living amoeba to elucidate the molecular mechanisms of encystation and excystation of pathogenic amoebae was an interesting outcome of this period. The work on the biochemistry of pathogenic microorganisms brought recognition in the shape of election to the Fellowship of the Indian National Science Academy and the Indian Academy of Sciences and the prestigious Basanti Devi Amirchand Award for outstanding biomedical research in 1973<sup>7-9</sup>.

### (5) Biochemical Toxicology

Our long-standing collaboration with King George's Medical College, Lucknow, bore fruit in the elucidation of the role of the skin of the neonate in facing the stress of physiological jaundice and the hyperbaric environment outside the womb. Collagen was shown to provide the matrix for binding bilirubin and exposing it to photodecomposition by light. The work was recognized by INSA in the award of the Sunder Lal Hora Medal for sustained contributions to the development of biological research<sup>10,11</sup>.

Transfer of my services from the Central Drug research Institute to the Industrial Toxicology Research Institute in 1975 enabled me to concentrate on problems related to the safety of industrial and agrochemicals<sup>12,13</sup>.

## (6) Post-Retirement

The Department of Environment, Government of India, has generously supported my current interest in ecotoxicological problems related to industrial and agrochemicals and the impact of development on River Basin ecosystems. I have also kept myself involved with the activities of the World Health Organization in the area of environmental pollution and human health.

The Madras Science Foundation commissioned me to write the second of the series of background reading material and the fruit of that labour—Environment, Chemicals and Man—is appearing shortly. The Department of Environment, Government of India, has been generous to associate me with the work of the Ganga Basin Ecodevelopment and the Integrated Environmental Programme on Heavy Metals. I have found these activities to be highly rewarding in that I can continue to interact with young scientists in our universities and research institutions and above all make me feel that the real journey of discovery is just about to begin.

## Selected Publications

1. Krishna Murti C R & Giri K V, Physiocochemical studies on papaya and Indian gooseberry, *Proc Indian Acad Sci*, **42B** (1950) 59.
2. Krishna Murti C R & Subrahmanyam V, Purification of the milk clotting enzyme of the fig latex, *Indian J Dairy Sci*, **6** (1953) 15.
3. Krishna Murti C R & Stone B A, Separation of glucanases, *Biochem J*, **77** (1961) 715.
4. Krishna Murti C R, Action of streptomycin on *V. cholerae*, *Biochem J*, **76** (1960) 362.
5. Krishna Murti C R, Iso-nicotinic acid hydrazide in antibiotics, Vol III, *Mechanism of action of antimicrobial and antitumor agents*, edited by J W Corcoran and F E Hahn (Springer Verlag, Berlin) 1974, 623.
6. Krishna Murti C R, The enzymology of *Vibrio cholerae*, *J scient ind Res*, **27** (1965) 430.
7. Raizada M K & Krishna Murti C R, Transformation of trophic *Hartmannella culbertsoni* into viable cysts by cyclic A M P, *J Cell Biol*, **52** (1972) 747.
8. Krishna Murti C R, Molecular biology of amoebic encystation, *Indian J med Res*, **63** (1975) 757.
9. Krishna Murti C R & Shukla O P, Differentiation of pathogenic amoebae encystation and excystation of *Acanthamoeba culbertsoni*, *J Biosci*, **6** (1984) 475-89.
10. Kapoor C L, Krishna Murti C R & Bajpai P C, Interaction of bilirubin with human skin, *New Engl J Med*, **188** (1973) 588.
11. Krishna Murti C R, Bilirubin—A model for the study of an endogenous toxic chemical, *Sunder Lal Hora Medal Lecture*, 1981.
12. *Biodegradable pesticides*, edited by F Matsumara and C R Krishna Murti (Plenum Press, New York) 1982.
13. Krishna Murti C R, *Status report of pesticide residues in biological tissues*, Indian National Science Academy 1985 Golden Jubilee Year, 1984.



## P K Maitra

My research has been concerned with control of energy-yielding processes in metabolism, in particular with biochemical and genetic regulation of carbohydrate metabolism. The methodological foundation of most of my later work was laid in the early sixties with the development of sensitive fluorescence assay of substrates and enzymes at the University of Pennsylvania<sup>1</sup>. This led to the chemical identification of respiratory control by ADP in yeast and to the rate-limiting steps in glycolysis in several systems. In the electric eel, for example, metabolite assays showed that the glycolytic pathway is the major source of energy during recovery from an electric discharge<sup>2</sup>. The idea of multi-site control of a pathway was developed while working with ascites tumour cells<sup>3</sup> and this eventually led to the discovery of glycolytic oscillations<sup>4,5</sup>.

At the Tata Institute of Fundamental Research, where I joined in 1963, I had started work on the genetic regulation of glycolysis in the bacterium *Escherichia coli* and later in the yeast *Saccharomyces cerevisiae*. Several features of the glycolytic pathway prompted these studies: the pathway is generally understood biochemically; however, at the time we started, very little was known about its genetic regulation. The pathway

is bidirectional and is constitutive. Is the synthesis of the pathway enzymes regulated, and if so, how? How would cell physiology be affected by mutations that render some of the allosteric enzymes of the glycolytic pathway non-allosteric? Are there biochemical alternatives to this pathway? How are the genes for the glycolytic enzymes disposed on the chromosome? How does one go about mutating these genes? Our work during the last 20 years has thrown some light on these questions.

In 1971, we showed that the glycolytic enzymes in the yeast are induced in response to the intracellular synthesis of glucose 6-phosphate<sup>6</sup>. This led to the idea that the synthesis of constitutive enzymes might require pools of internally generated inducers. A method of isolating glycolytic mutants was developed<sup>7</sup>. Mutations in most of the dozen enzymes of the glycolytic pathway were obtained in *E. coli* and in yeast. Genetic and biochemical characterization of these mutations showed that the pathway has really no metabolic alternative; however, several reactions are catalyzed by multiple enzymes. A typical example is the phosphorylation of glucose for which three genes were found<sup>8</sup>. As a rule, these genes were disposed all over the chromosomes both in *E. coli* and in yeast<sup>9</sup>. However, no

---

Professor of Molecular Biology, Tata Institute of Fundamental Research, Colaba, Bombay-400005;  
Residence : 608, TIFR Housing Colony, Colaba, Bombay-400005.

evidence of any regulator gene was obtained.

Two new enzymes were discovered, one a glucokinase that phosphorylates glucose at the 6-position<sup>10</sup> and the other a particulate variety of an isozyme of fructose 6-phosphate kinase<sup>11</sup> distinct from the soluble, allosteric one. The genetic study of the particulate enzyme indicated that it shares a common gene *PFK2* with the soluble enzyme. While for the particulate enzyme this gene specifies a subunit essential for catalysis, for the soluble enzyme it specifies the regulatory subunit that confers on the latter the property of inhibition by ATP; the catalytic subunit of the soluble enzyme is specified by another unlinked gene, *PEK1*<sup>12,13</sup>.

Surprisingly, the loss of regulatory control by mutation in two allosteric enzymes of the glycolytic pathway, viz. soluble phosphofructokinase and pyruvate kinase<sup>14</sup>, does not confer on the yeast cell any discernible derangement of function in either glycolysis or gluconeogenesis.

### Selected Publications

1. Maitra P K & Estabrook R W, A fluorometric method for the enzymic determination of glycolytic intermediates, *Anal Biochem*, **7** (1964) 472-84.
2. Maitra P K, Ghosh A, Schoener B & Chance B, Transients in glycolytic metabolism following electrical activity in electrophorus, *Biochim biophys Acta*, **88** (1964) 112-19.
3. Maitra P K & Chance B, ADP and Pi control in ascites tumor cells, in *Control of energy metabolism*, edited by B Chance, R W Estabrook and J R Williamson (Academic Press, New York) 1965, 157-75.
4. Chance B, Ghose A, Schoener B & Maitra P K, Cyclic and oscillatory responses of metabolic pathways involving chemical feedback and their computer representations, *Ann N Y Acad Sci*, **115** (1964) 1010-24.
5. Maitra P K, Pulsating glucose flux in yeast, *Biochem biophys Res Commun*, **25** (1966) 462-67.
6. Maitra P K & Lobo Z, Control of glycolytic enzyme synthesis in yeast by products of the hexokinase reaction, *J Biol Chem*, **246** (1971) 489-99.
7. Irani M & Maitra P K, Isolation and characterization of *Escherichia coli* mutants defective in enzymes of glycolysis, *Biochem biophys Res Commun*, **56** (1974) 127-33.
8. Lobo Z & Maitra P K, Genetics of yeast hexokinase, *Genetics*, **86** (1977) 727-44.
9. Maitra P K, Highlights of glycolysis genetics, *Indian J Microbiol*, **20** (1980) 329-32.
10. Maitra P K, A glucokinase from *Saccharomyces cerevisiae*, *J Biol Chem*, **245** (1970) 2423-31.
11. Lobo Z & Maitra P K, A particulate phosphofructokinase from yeast, *FEBS Lett*, **137** (1982) 279-82.
12. Lobo Z & Maitra P K, Genetic evidence for distinct catalytic and regulatory subunits in yeast phosphofructokinase, *FEBS Lett*, **139** (1982) 93-96.
13. Lobo Z & Maitra P K, Phosphofructokinase mutants of yeast : Biochemistry and genetics, *J Biol Chem*, **258** (1983) 1444-49.
14. Maitra P K & Lobo Z, Yeast pyruvate kinase : A mutant form catalytically insensitive to fructose 1,6-bisphosphate, *Eur J Biochem*, **78** (1977) 353-60.



## N R Moudgal

Advances in our knowledge of endocrinological processes has periodically received a great boost by the development of newer methods to study specific events. Among these, mention should be made of classical advances like (i) perfection of methods of extirpate specific glands in different species of animals, (ii) identification, isolation, characterization and in many cases, synthesis of hormones, (iii) development of sensitive methods for the assay of hormones like radioimmuno- and radioreceptor assays, and (iv) development of methods to study the mechanism of action of hormones at the physiological and cellular levels.

Significant contribution has been made by Moudgal's group over the last 2 years to evolve methods to study the action of gonadotropins at the whole animal and cellular levels. A classical method to obtain the deficiency of a pituitary hormone is by hypophysectomy, but this leads in addition to deprivation in the hormone one is interested in, the deficiency of several other key hormonal factors, and consequently it is virtually impossible to mimic by restitution therapy hormonal interplay that occurs in nature with respect to dosage, duration and sequelae. The usage of characterized antisera (a/s) of high specificity of individual hormones, such as Luteinizing (LH), Follicle

Stimulating (FSH) hormones and human chorionic gonadotropin (hCG) has been championed by Moudgal as a simple but specific means of inducing deficiency of only the desired hormone for specific durations. Since the levels of hormones other than the one that is neutralized with the specific antibody is not disturbed in this method it will permit studying the effect of depriving an animal in a near normal physiological state a single hormonal entity. Although most of the studies carried out by his group pertain to usage of polyclonal antibodies, rigorous methods have been evolved by them to render these antisera mono-specific with respect to a given hormone.

Fundamental contributions made by Moudgal and his coworkers towards the understanding of specific events in the reproductive biology of the mammal, including the nonhuman primate, can be categorized as follows:

- (1) Understanding the relative role of FSH and LH in the cyclic follicular maturation and ovulation in the rat, hamster and the monkey.
- (2) Understanding the mechanism of luteal function by LH.
- (3) Establishing the critical need for LH/CG to maintain early pregnancy in the monkey.

(4) Understanding the mechanism of LH and FSH action at the cellular and molecular level.

(5) Attempt at understanding the mechanism of ovarian quiescence brought about by lactation in the adult male nonhuman primate.

(6) Establishing a role for FSH in the maintenance of spermatogenesis and fertility in the adult male nonhuman primate.

(7) Studies on the isolation and characterization of an FSH suppressing factor (Inhibin) from the testes.

(8) Studies on hormonal regulation of implantation in the primate.

### Selected Publications

1. Madhwaraj H G & Moudgal N R, Hormonal control of gestation in the intact rat, *Endocrinology*, **86** (1970) 874-89.
2. Moudgal N R, Macdonald C J & Greep R O, Effect of human chorionic gonadotropin (HCG) antiserum on ovulation and corpus luteum function in the monkey *M. fascicularis*, *J clin Endocr Metab*, **32** (1971) 579-81.
3. Moyle W R, Moudgal N R & Greep R O, Cessation of steroidogenesis in leydig cell tumors after removal of luteinizing hormone and adenosine cyclic 3', 5'-monophosphate, *J biol Chem*, **246** (1971) 4978-82.
4. Moudgal N R, Moyle W R & Greep R O, Specific binding of luteinizing hormone to leydig tumor cells, *J biol Chem*, **46** (1971) 4983-86.
5. Moudgal N R, Macdonald G J & Greep R O, Role of endogenous primate LH in maintaining corpus luteum of the monkey, *J clin Endocr Metab*, **35** (1972) 113-16.
6. Moudgal N R, Behrman H R & Greep R O, Effect of LH antiserum (a-s) on progesterin secretion from the pregnant rat ovary, *J Endocr*, **52** (1972) 413.
7. Moudgal N R, Role of LH in maintenance of luteal function, *Proc Int Cong in Endocrinology*, Washington D C (Excerpta Medica) 1973.
8. Moudgal N R, Jagannadha Rao A, Maneckjee R, Muralidhar K, Mukku V R & Sheela Rani C S, Gonadotropins and their antibodies, *Rec Prog Horm Res*, **30** (1974) 47.
9. Moudgal N R, Hormone antibodies—An appraisal of their use in reproductive endocrinology, in *MTP Int Review of Science Physiology, Series 1, Reproductive physiology*, Vol 8 (Butterworths & Co. Ltd, London), 1974, 33.
10. Mukku V R & Moudgal N R, Studies on luteolysis—Effect of LH antiserum on sterol and steroid levels in pregnant hamster, *Endocrinology*, **97** (1975) 1455-59.
11. Muralidhar K & Moudgal N R, Studies on rat ovarian receptors to luteinizing hormone—Factors influencing binding and response, *Biochem J*, **160** (1976) 607-13.
12. Muralidhar K & Moudgal N R, Studies on rat ovarian receptors to luteinizing hormone—interaction of B-subunit of LH, *Biochem J*, **160** (1976) 615-19.
13. Maneckjee R, Sreenath B R & Moudgal N R, Prolactin suppresses LH release during lactation in the monkey, *Nature, Lond*, **262** (1976) 507-8.
14. Moudgal N R, Mukku V R, Prahalada S, Murty G S R C & Li C H, Passive immunization with an antibody to the B-subunit of ovine luteinizing hormone as a method of early abortion—A feasibility study in monkeys (*Macaca radiata*), *Fertil Steril*, **30** (1978) 223-29.
15. Sheela Rani C S & Moudgal N R, Examination of the role of follicle stimulating hormone in estrogen biosynthesis *in vivo* and *in vitro* in the ovary of cyclic hamster, *Steroids*, **32** (1978) 435-51.
16. Moudgal N R, A need for FSH in maintaining fertility of adult male subhuman primates, *Arch Androl*, **7** (1981) 117-25.
17. Moudgal N R & Li C H, B-subunits of human choriogonadotropin and ovine lutropin are biologically active, *PNAS*, **79** (1982) 2500-3.
18. Moudgal N R & Sheela Rani C S, Advances in immunobiology of gonadotropins, in *Hormonal proteins & peptides*, Vol II (Academic Press Inc, New York) 1983, 135-84.
19. Dhanasekaran N, Sheela Rani C S & Moudgal N R, Studies on follicular atresia: Lysosomal enzyme activity and gonadotropin receptors of granulosa cells following administration or withdrawal of gonadotropins in the rat, *Molec Cell Endocr*, **33** (1983) 97-112.
20. Dighe R R & Moudgal N R, Use of  $\alpha$  and  $\beta$  subunit specific antibodies in studying interaction



- of hCG with Leydig cell receptors, *Archs Biochem Biophys*, **225** (1983) 490-99.
21. Moudgal N R, Corpus luteum of the nonhuman primate, in *Adv in Vet Sci & Comparative Med*, Vol 28 (Academic Press Inc., New York) 1984, 343-66.
22. Moudgal N R & Rao A J, Contraception through immunization and regulation of luteal function, in *Human fertility, health and food: Impact of molecular biology and biotechnology*, edited by David Puett (United Nations Fund for Population Activities, New York) 1984, 73-79.
23. Sheela Rani C S & Moudgal N R, Differences in the behaviour of luteinizing hormones of various species at the rat gonadal cell receptor site, *Endocrinology*, **116** (1985) 597-603.
24. Moudgal N R, Maneckjee R, Srinath B R & Ramasharma K, Studies on ovarian quiescence in the lactating bonnet monkey (*Macaca radiata*), *J Biosci*, **7** (1985) 95-103.
25. Ravindranath N & Moudgal N R, Antifertility effect of tamoxifen as tested in the female bonnet monkey (*Macaca radiata*), *J Biosci*, **10** (1986) 167-70.

## M C Nath

During his research career as the Founder Professor and Head, Department of Biochemistry, Nagpur University, spanning over almost a quarter of a century (1946-71), Nath made fundamental contributions from his studies on metabolic disorders like diabetes, hypertension and atherosclerosis.

### Diabetes

It was reported<sup>1</sup> from Nath's laboratory in 1944 that injection of acetoacetate or  $\beta$ -hydroxybutyrate causes an immediate rise in the fasting blood sugar levels of normal rabbits and their continued daily injection gives rise to a condition of hyperglycemia followed by an initial hypoglycemic state<sup>2</sup>, which was thought to be due to the stimulated insulated secreting mechanism by these ketone bodies. It was reported later how acetoacetate and  $\beta$ -hydroxybutyrate can inactivate the effect of insulin both *in vitro* and *in vivo*. Confirmatory evidence was obtained from assay of insulin contents of pancreas of guineapigs given a daily dose of these ketone bodies for several weeks; a marked initial increase in the insulin content was noted for 2-4 weeks, but it fell to the extent of 50% of the normal level after 70 days<sup>4,5</sup>.

It was shown later<sup>4</sup> that daily injection of acetoacetate in gradually increasing doses could bring about a condition of

progressive decreased glucose tolerance and glycogen storage in a large number of animals (rabbits) kept on horse gram (*Cicer arietinum*) diet, accounting for the increased fasting blood sugar level, which is observed in men placed on high fat diet<sup>5</sup>.

From another study, Nath and coworkers<sup>5</sup> showed that acetoacetate induced diabetes in rabbits can be completely prevented by supplementing either 2% methionine or 26% casein to the sulphhydryl deficient diet. With regard to the correlation between thiamine deficiency and diabetes, Nath and Chakrabarti<sup>6</sup> observed that continued injections of acetoacetate cause depletion of thiamine both *in vitro* and *in vivo* and there is marked reduction in the riboflavin and niacin contents of the blood of the treated animals<sup>7</sup>. An enormous rise in the pyruvic acid content of the blood was also observed in the acetoacetate injected animals. Later work by Nath and Shastri<sup>8</sup> indicated that there is also a depletion of vitamin B<sub>6</sub> in blood and tissues of rats injected with acetoacetate. That the ketone bodies on prolonged administration can bring about depletion of vitamin B<sub>12</sub> in blood and tissues of animals was shown by Nath and Nath<sup>9</sup>.

With regard to the effect of acetoacetate on blood GSH level, Nath and Hatwalne<sup>10</sup> showed that a single massive

---

Formerly, Professor & Head, Department of Biochemistry, University of Nagpur, Nagpur; Residence : 8 East High Court Road, New Ramdaspath, Nagpur-440010.



subcutaneous injection of sodium acetoacetate brings about a lowering of the reduced glutathione content of the blood by 30-60%, depending upon the dose used. In a subsequent study, Nath *et al.*<sup>11</sup> observed that injection of 300 mg/kg of acetoacetate in normal rabbits depletes the GSH content of blood to the level of 6 mg/100 ml after 1 h of each injection. Fall in GSH was found to be accompanied by a rise in blood sugar.

Nath and Chakrabarti<sup>12</sup>, from studies on acetoacetate induced rise in blood lactic acid in rabbits on gram diet, noticed a fall in the ascorbic acid content in the blood. Later, Nath *et al.*<sup>13</sup> reported aggravation in the disturbance of carbohydrate metabolism accompanied by a marked depletion of liver and muscle glycogen in guineapigs denied ascorbic acid following repeated injection of acetoacetate. A still more interesting observation<sup>14</sup> was the accumulation of dehydroascorbic acid and greater depletion of ascorbic acid in the various tissues of the accetoacetate treated guineapigs denied ascorbic acid.

With regard to the metabolic relationship between acetoacetate and glucose, it was reported from Nath's laboratory<sup>15</sup> that although glucose or acetoacetate when injected individually to the rabbits on gram diet causes immediate rise in the blood sugar level, when the two substances are injected simultaneously or the condensation product of the two is injected into the animals, there is no appreciable rise in blood sugar at all. Such metabolic relationship between glucose and acetoacetate reveals the possibility of another pathway of glucose utilization. This finding lent support to the hypothesis put forward by Nath<sup>16</sup> and some other

workers that the rise in blood sugar in diabetes may, to a great extent, be the result of a physiological response and may, therefore, be considered as a protective phenomenon.

### Hyperlipemia and Atherosclerosis

With regard to the role of cholesterol in atherosclerosis, Nath and Saikia<sup>17</sup> reported that feeding of high saturated fat diets containing margarine or dalda (hydrogenated vegetable oil) not only brings about an increase in the levels of total and ester cholesterol in the plasma and tissues of animals but also raise the total cholesterol/lipid phosphorus (C/P) ratio to a considerable extent. It was also shown by them that hydrolysed glucose cycloacetoacetate (GCA) when injected daily to such high fat fed animals in the dose of 80 mg/100 g body weight checks such increase in cholesterol level and C/P ratio. From their attempt to study the physiological significance of inositol, vitamin B<sub>12</sub> and hydrolyzed GCA in cholesterol-induced atherosclerosis in rabbits, Nath and Saikia<sup>18</sup> observed that hydrolyzed GCA has got an almost similar effect as vitamin B<sub>12</sub> and the effect is much higher with GCA (hydrolyzed). The increase in the body weight is also highest in the animals injected with GCA (hydrolyzed).

Nath and coworkers made notable contributions with regard to the effects of essential fatty acids, vitamin B<sub>12</sub> and hydrolyzed GCA on saturated fat-induced atherosclerosis. Nath and Saikia<sup>19</sup> observed that on feeding hydrogenated vegetable fat to rabbits, the serum cholesterol level and the C/P ratio were much higher compared to those in animals kept either on linseed oil or a mixture of

essential fatty acids. The elevated serum and tissue lipid levels and C/P ratio of the animals kept on hydrogenated vegetable fat were reduced considerably when the animals were injected with GCA (hydrolyzed) even to a greater extent compared to animals kept on dalda and linseed oil. The effect of essential fatty acid mixture in maintaining the normal serum and tissue lipid levels and their C/P ratios was markedly pronounced when the animals were injected either with GCA (hydrolyzed) or vitamin B<sub>12</sub>. Maximum gain in body weight was noted in the case of unsaturated fat diet and also in the case of the animals kept on hydrolyzed GCA along with essential fatty acid supplemented dalda diet.

To investigate whether experimental atherosclerosis induced by a diet containing highly saturated fat is associated with change in coagulation time, prothrombin time and the platelet content of blood, Nath and Saikia<sup>20</sup> studied the effects of essential fatty acids, vitamin B<sub>12</sub>, inositol and hydrolyzed GCA on these factors in saturated fat-induced atherosclerotic animals. They found that prolonged feeding of a diet containing 20% of highly saturated fat to rabbits, which leads to experimental atherosclerosis, shortens the coagulation factors (bleeding time, coagulation time and prothrombin time) and increases liver fat and the number of blood platelets and the ester cholesterol level in blood and heart. It was suggested that the rise in tissue ester cholesterol, especially of the superficial intima layers may activate blood platelets, thus leading to increased coagulability of blood and thrombosis. The administration of essential fatty acids (linoleic and linolenic), inositol, vitamin B<sub>12</sub> and hydrolyzed GCA was shown to counteract

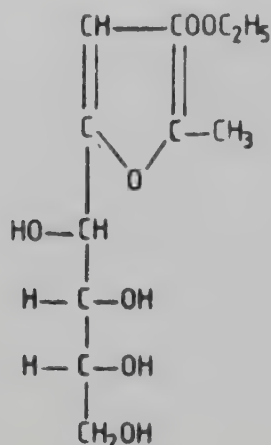
such shortening of coagulation factors. Nath and Saikia<sup>20</sup> further found that although continued use of saturated fat causes a decrease in the tissue phospholipids, their concentration in the serum is very much elevated, which is responsible for activating the platelets and thus shortening the blood coagulation time. Essential fatty acids, inositol, vitamin B<sub>12</sub> or hydrolyzed GCA, when administered along with the saturated fat diet, were found to increase tissue phospholipid and to decrease its release in the serum as well as to check the rise in blood platelets.

From their studies aimed at finding a cure for experimental atherosclerosis, Nath and Saikia<sup>20</sup> observed that when rabbits induced hyperlipaemic by feeding saturated fat (dalda) diet and cholesterol were treated with essential fatty acids, vitamin B<sub>12</sub>, dl-methionine or hydrolyzed GCA, the elevated C/P ratio of these animals began to fall rapidly. It was also observed that the effect of hydrolyzed GCA in lowering the C/P ratio is more pronounced than that of either vitamin B<sub>12</sub>, dl-methionine or essential fatty acids. On administration of linseed oil along with hydrolyzed GCA, the atherosclerotic animals showed the maximum curative effect, as indicated by the lowering of the C/P ratio, bringing it almost to the normal level in about 8 weeks' time. These results indicate that hydrolyzed GCA is an active factor in accelerating the therapeutic effect of linseed oil or essential fatty acids in curing experimental atherosclerosis by raising the antilipaemic effect either through decreased formation of cholesterol or its increased excretion of faecal bile acids.

GCA was prepared by West in 1927 by condensing glucose with ethyl

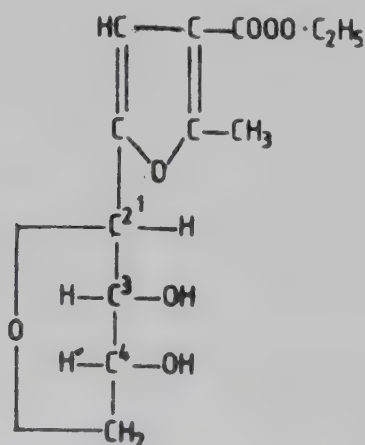


acetoacetate, but he did not think of any biochemical properties of the substance [*J biol Chem*, **74** (1927) 561]. Its structure was determined by Gonzales and Aparatio in 1955 [*Ann Fioquim*, **41** (1958) 436], which is as follows:



While studying the effect of GCA on the biosynthesis of vitamin C, Nath and coworkers<sup>21</sup> modified the method of its preparation in 1953 to give better yield.

The structure of GCA (h) as determined of Yamazaki of Tokyo (private communication) was as follows:



It can be termed as 2-(2<sup>1</sup>-3<sup>1</sup>-4 dihydroxytetrahydrofuryl, 5-methyl, 4-carbothoxyfuran (or F F). Yamazaki also

confirmed some of the findings of Nath and coworkers. It is an oily substance having B P 205°C and (n)<sub>D</sub>, 87.1°. It is very labile to sunshine and heat. This was formerly called GCA(h) by Nath and his associates. Compounds GCA and F F had been previously shown by Nath and his associates to be helpful not only in preventing the onset of experimental diabetes of various kinds and the onset of atherosclerosis by food factors, but also in curing experimental diabetes and experimental atherosclerosis to a great extent.

The influence of F F on cholesterol metabolism and biosynthesis of bile acids was recently studied by Yamazaki with <sup>14</sup>C-mevalonic acid on bile fistula rats and the following results were observed: (1) Cholesterol biosynthesis is not influenced by F F, (2) biliary excretion of cholesterol was slightly increased, and (3) biosynthesis of bile acids from cholesterol was promoted.

These findings have confirmed the report of Nath on the beneficial role of GCA(h) or F F in atherosclerotic condition in animals as evidenced by decreases in the C/P ratio in blood and tissues in animals and rise in the blood clotting times in human patients.

### Pharmacological and Toxicity Studies

(a) It was shown that oral administration of GCA and GCAh to young albino mice does not cause acute toxicity even in high doses. Doses up to 250 mg/100 g body weight were found to be absolutely non-toxic; LD<sub>50</sub> of GCAh is 600 mg/100 g body weight<sup>22</sup>.

(b) The work on sub-acute toxicity has shown that ingestion of GCA or GCAh to weanling albino rats at levels as high as

10% of the diet, for a period of 90 days, does not alter their normal growth in any way. The mortality among test rats was not increased and organ weights were not significantly altered from those of the normals. There was no alteration in the physiological function, as determined by urine analysis or haematologic study. On the other hand, presence of GCA and GCAh in the diet appeared to improve the efficiency of food utilization. Prolonged intravenous injection of GCA and GCAh has been found to cause a slight increase in haemoglobin level, erythrocyte count and packed cell volume<sup>23</sup>.

The effect of GCA(h) on vitamin B<sub>12</sub> activity in E. Leichanni-4794 was observed by Nath and Nath<sup>24</sup>.

Apart from reporting the beneficial roles of GCA and GCA(h) in carbohydrate and lipid metabolism, Nath and coworkers also studied their roles in the biosynthesis of vitamin C, niacin and riboflavin in germinating mung beans and in the intestine.

The roles of these compounds in anaemia, protein metabolism, etc. and their effect on glutathione levels in blood were also studied in Nath's laboratory.

## Selected Publications

1. Nath M C & Brahmachari H D, Experimental hyperglycemia by injection of intermediary fat metabolism products in rabbits, *Nature, Lond*, **154** (1944) 487.
2. Nath M C & Brahmachari H D, Studies on the aetiological factors responsible for the onset of diabetic symptoms. Part I, *Indian J med Res*, **37** (1949) 61.
3. Nath M C & Brahmachari H D, Inactivation of insulin by intermediary fat metabolites, *Nature, Lond*, **157** (1946) 336.
4. Nath M C & Chakrabarti C H, Progressive decreased glucose tolerance and glycogen storage following acetoacetate injection, by insulin, *Proc Soc exp Biol Med*, **75** (1950) 326.
5. Nath M C, Chakrabarti C H & Nayudu S G, Effect of methionine and casein on acetoacetate induced hyperglycemia, *Proc Soc exp Biol Med*, **88** (1955) 416.
6. Nath M C & Chakrabarti H C, Studies on the effect of acetoacetate and  $\beta$ -hydroxy butyrate on vitamin B<sub>1</sub> both *in vivo* and *in vitro*, *Indian J med Res*, **31** (1953) 257.
7. Nath M C & Chakrabarti H C, Effect of acetoacetate and  $\beta$ -hydroxybutyrate on vitamin B<sub>2</sub> and nicotinic acid, *Proc Soc exp Biol Med*, **82** (1953) 5.
8. Nath N, Sivakumar B & Nath M C, Effect of acetoacetate administration on urinary and tissue vitamin B<sub>6</sub> in rats, *Br J Nutr*, **23** (1969) 705.
9. Nath N & Nath M C, Effect of acetoacetate and  $\beta$ -hydroxy butyrate on vitamin B<sub>12</sub> in rats, *Proc Soc exp Biol Med*, **124** (1967) 210.
10. Nath M C & Hatwalne V G, Effect of acetoacetic acid on reduced glutathione content of blood in rabbits, *Nature, Lond*, **160** (1950) 692.
11. Nath M C, Hatwalne V G & Gadgil J S, Progressive depletion in the reduced glutathione content of the blood following acetoacetate injection, *Biochem J*, **53** (1953) 479.
12. Nath M C & Chakrabarti C H, Acetoacetate induced changes in blood lactic acid and ascorbic acid and their prevention by insulin, *Proc Soc exp Biol Med*, **78** (1951) 369.
13. Nath M C, Sahu V K & Chitale R P, Role of acetoacetate in aggravating the disturbances in the carbohydrate metabolism of scorbutic guinea pigs, *Biochem J*, **53** (1953) 684.
14. Behki R M, Rajdan M & Nath M C, Effect of acetoacetate on GSH and dehydroascorbic acid of the tissues of scorbutic guinea pigs, *J scient ind Res*, **16C** (1957) 193.
15. Nath M C & Sahu V K, Metabolic relationship between acetoacetate and glucose, *Proc Soc exp Biol Med*, **79** (1952) 608.
16. Nath M C, Recent ideas on the cause, theory and treatment of diabetes, *Sci & Cult*, **12** (1947) 390.
17. Nath M C & Saikia A, Studies on lipid metabolism. 1. Effect of different food fats on cholesterol/lipid (C/D) phosphorus ratio in plasma and tissues of rats and the role of glucose cycloacetoacetic acid on this ratio, *Indian J med Res*, **46** (1958) 579.
18. Nath M C & Saikia A, Effect of inositol, vitamin B<sub>12</sub> and glucose cycloacetoacetate on cholesterol



- induced atherosclerosis on rabbits, *Archs Biochem Biophys*, **79** (1959) 216.
19. Nath M C & Saikia A, Effect of different food fats on experimental atherosclerosis and the beneficial role of essential fatty acids, vitamin B<sub>12</sub> and glucose cycloacetoacetate (hydrolysed), *Archs Biochem Biophys*, **84** (1959) 167.
  20. Nath M C & Saikia A, Physiological role of essential fatty acids, inositol vitamin B<sub>12</sub> and glucose cycloacetoacetate (hydrolysed) on blood coagulation factors of saturated fat induced atherosclerosis in rabbits, *Am J Nutr*, **69** (1959) 403.
  21. Nath M C, Chitale R P & Bhavani Belavady, Studies on the synthesis of vitamin C, a new precursor, *Nature, Lond*, **170** (1953) 53.
  22. Dorle & Nath M C, Studies on acute toxicity of glucose cycloacetoacetate and its hydrolyzed products, *Indian J Physiol all Sci*, **16** (1962) 4.
  23. Dorle & Nath M C, The sub-acute toxicity of glucose cycloacetoacetate and its hydrolysate, *Indian J Physiol all Sci*, **19** (1965) 25.
  24. Nath M C & Nath N, Effect of GCA(h) on vitamin B<sub>12</sub> activity in E Leichmanni-4794, *J Vitaminol*, **15** (1969) 208.

## N K Notani

### Genetic Organization in Plants

My early contributions are in genetical research with plants (mostly maize). Back in the 1950's, when Barbara McClintock first encountered *controlling elements* in maize she distinguished them from genes. She took the conceptual leap and deduced that these were transposable. However, their role in regulation or development was intriguing. In the 1960's, when Jacob and Monod proposed their operon model of coordinate regulation of genes in bacteria, McClintock drew parallels between controlling elements and (transposed) operators. We tested this hypothesis for the *Ds-Ac* system. If *Ds* element was a transposed operator sitting next to a gene, we argued that it should be possible to mutate or inactivate it. The results were negative with the known mutagenic agents<sup>1</sup>. From our observations we proposed an excision model which is somehow bearing out. With the development of gene-cloning techniques, a number of insertions and their excision have been analyzed. The (*Ds*) insertions seem to be heterogeneous with regard to size. We have recently isolated a cob from the local market which shows somatic instability. Our interest is to see if the Indian strain harbours any of the known transposable elements, which may throw light on the natural history of these elements.

In the 1960's, R A Brink reported a phenomenon in maize, which has been noted in a few other species. Paramutation, as he termed it, is observed when *R* (self-cloned aleurone) allele is extracted from an  $R R^{st}$  ( $R^{stippled}$ ) heterozygote. *R* expression is markedly reduced and it occurs as a mass phenomenon. Moreover, the reduced expression has some heritability. I worked with Brink for a short period during which we analyzed the effects of certain chromosomal translocations on the expression of *R* gene and we arrived at the conclusion, then altogether novel, that gene loci must be larger than had been generally assumed till then<sup>2</sup>.

### (2) Mechanism of Genetic Transformation in Bacteria

The work of Avery *et al.* (1944) showed that transforming principle is DNA. The generalization that all (primary) genetic material is DNA took another 7 or 8 years. Towards the end of the 1950's, the technique of equilibrium density-gradient centrifugation was worked out and was used in early 1960's to elucidate the physical nature of genetic recombination. In the mid-1960's we applied this technique in *Haemophilus influenzae*, an excellent transformation system. We found that transformation occurs by insertion of single-strand segments of donor DNA

---

Associate Director, Biomedical Group & Head, Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay-400085; Residence : 10B, Department of Atomic Energy Apartments, 317 Ridge Road, Bombay-400006.



displacing the resident DNA homologous segment<sup>3</sup>. Later, we showed that either strand of DNA can transform<sup>4</sup>. In early 1970's, I analyzed the intracellular donor DNA and showed that part of the donor DNA after entry is present as fragments which sedimented slower than input DNA and had poor biological activity. I called them species II molecules<sup>5</sup>. We developed here a method of digitonin lysis which allowed easy separation of donor DNA from the resident DNA. We used this method to analyze two strains (*rec1* and *rec2*) both deficient in genetic recombination. We showed that these are blocked in two different steps both by the digitonin method and also by equilibrium density-gradient centrifugation<sup>6</sup>. We also did a transfection study in which we showed that phage DNA after entry fragments, but is reassembled by recombination to form concatenes<sup>7</sup>.

In the 1970's, gene cloning systems began to be developed. Using a multicopy plasmid RSF 0885, we also developed a cloning system which had some very novel features. RSF 0885 by itself transformed very poorly, a fact explained on the basis of H O Smith's idea of the presence of uptake sequences<sup>8</sup>. For chimeric plasmids, there was a requirement of *rec* gene expression<sup>9,10</sup>. We then constructed a super-vector, which permitted selection of clones with great ease<sup>11</sup>. Using this vector, several *nov<sup>r</sup>* gene clones were obtained<sup>12</sup>. These have provided illuminating insights into transformation with chimeric plasmid DNA. Our current and future interest is in cloning of genes for DNA metabolism.

### (3) DNA Repair and Mutagenesis

Bacterium *H. influenzae* has certain features which are different from those of *E. coli*. *H. influenzae* is not mutable by UV

light, PUVA (psoralen + UVA) or MMS (methylmethanesulfonate)<sup>13-15</sup>. One explanation that we have offered is that it lacks the counterpart of *umu* (UV-mutability) genes of *E. coli*. DNA repair of PUVA-treated cells also has some differences from the same in *E. coli*. *H. influenzae* Rec1 strains are not as sensitive as RecA of *E. coli*. Also, whereas induction of prophage with PUVA is possible, it is not blocked by chloramphenicol. Similarly, W-reactivation (Weigle-reactivation) is observed, but the surviving phage is not mutated<sup>16</sup>. We have recently cloned a DNA repair gene *uvr1* (on pKuvr 1), which specifically complements a *uvr1* mutation, but not a *uvr2* mutation. With locus-directed mutagenesis of this clone (pKuvr1), three new mutations affecting SOS phenotypes have been isolated<sup>17</sup>.

### (4) Rhizobium Plasmids

There has been enormous interest in megaplasmids of rhizobia, because they carry at least some of the *nif* and *nod* genes. In the process of analyzing *R. meliloti* isolated from *methi* (*Trigonella foenum-graecum*), we encountered very small plasmids ( $6 \times 10^6$  Mr). Plasmids smaller than  $1 \times 10^7$  Mr have been reported for the first time<sup>18</sup>. This plasmid is apparently cryptic, but we are trying to put markers which would enable us to use it possibly as a vector for making transfers in plants. Also, we are trying to determine their origin—whether they have originated from the bacterial chromosome or from a large plasmid or they have been 'added' from outside.

### Selected Publications

1. Chandra Mouli & Notani N K, Absence of detectable change in Ds at A1 locus in maize following mutagenic treatments, *Can J Genet Cytol*, **12** (1970) 436-42.

2. Brink B A & Notani N K, Effect of *R'* action in maize of a structural alteration distal to the *R* locus in chromosome 10, *Genetics*, **46** (1961) 1226-30.
3. Notani N K & Goodgal S H, On the nature of recombinants formed during transformation in *Haemophilus influenzae*, *J gen Physiol*, **46**(Pt 2) (1966) 197-209.
4. Godgal S H & Notani N K, Evidence that either strand of DNA can transform, *J molec Biol*, **35** (1968) 449-53.
5. Notani N K, Genetic and physical properties of unintegrated donor DNA molecules during *Haemophilus* transformation, *J molec Biol*, **59** (1971) 223-26.
6. Notani N K, Setlow J K, Joshi V R & Allison D P, Molecular basis for the transformation defects in mutants of *Haemophilus influenzae*, *J Bact*, **110** (1972) 1171-80.
7. Notani, N K, Setlow J K & Allison D P, Intracellular events during infection by *Haemophilus influenzae* phage and transfection by its DNA, *J molec Biol*, **75** (1973) 581-99.
8. Notani N K, Setlow J K, McCarthy D & Clayton N L, Transformation of *Haemophilus influenzae* by the plasmid RSF 0885, *J Bact*, **148** (1981) 812-16.
9. Notani N K, Genetic and molecular events in transformation of *H. influenzae* with plasmid RSF 0885 carrying shotgunned segments of chromosomal DNA, *J Biosci*, **3** (1981) 431-38.
10. Setlow J K, Notani N K, McCarthy D & Clayton N L, Transformation of *Haemophilus influenzae* by the plasmid RSF 0885 containing cloned segments of chromosomal DNA, *J Bact*, **148** (1981) 11.
11. Joshi V P & Notani N K, A new DNA-cloning vector for *Haemophilus influenzae* Rd, *J Biosci*, **5** (1983) 339-45.
12. Joshi V P & Notani N K, Cloning of *nov'* gene in *Haemophilus influenzae*, *Indian J exp Biol*, **22** (1984) 625-28.
13. George M & Notani N K, Induction of prophage and absence of mutation induction by psoralen plus near UV light exposure in *Haemophilus influenzae*, *Indian J exp Biol*, **18** (1980) 580-82.
14. Notani N K & Setlow J K, Inducible repair system in *Haemophilus influenzae* unaccompanied by mutation, *J Bact*, **143** (1980) 516-19.
15. Joshi V P, Bajaj M H, George M F, do Rego M H & Notani N K, Nature of radiation and chemically-induced lesions and role of cellular mechanisms in cell survival and mutagenesis, in *Advances in Biological and Medical Physics*, Vol 17 (Academic Press, New York and London) 1980, 115-28.
16. George M R & Notani N K, Genetic control of prophage induction in *Haemophilus influenzae* after exposure to psoralen near UV light, *J Virol*, **36** (1980) 965-67.
17. Kanade R P, Notani N K, Self-cloning in *Haemophilus influenzae* of *uvr1* gene, *Proc Symp Newer Approaches to Biological Applications*, 1984, 141-43.
18. D'Souza M R & Notani N K, Isolation and characterization of small plasmids from *Rhizobium meliloti*, *Curr Sci*, **53** (1984) 186-88.



## G Padmanaban

Iron is essential for the survival of the living cell. Heme is a major functional form of iron. It is well known and obvious that heme is essential for the structural integrity and functional activity of hemoglobin, cytochromes and other hemoproteins. Research in my laboratory during the last 15 years has clearly established that heme can regulate a variety of steps, including transcription, translation, protein transport and stabilization. Many other laboratories in the world are looking at the involvement of heme in one or the other of the vital processes in a living cell.

### Regulation of Heme Biosynthesis

Heme is a unique metabolite in that the first ( $\delta$ -aminolevulinate synthetase) and the last enzymes (heme synthase) of its own biosynthesis are located in mitochondria. The other steps are located in the cytosol. The final product, heme, is made in mitochondria and is then distributed to a variety of proteins in which it serves as a prosthetic group. It was no surprise when we found that heme regulates its own synthesis. Our early studies with *Neurospora* revealed that  $\delta$ -aminolevulinate dehydratase, the second enzyme of the pathway, is subjected to feedback repression by protoporphyrin, the penultimate end product. However, this enzyme constitutes the first enzyme and

protoporphyrin the end product, in the cytosolic segment of the heme-biosynthetic pathway. Ultimately, this regulation was found to encompass yeasts and fungi<sup>1,2</sup>.

In the animal system, the most important regulatory enzyme of the heme-biosynthetic pathway is  $\delta$ -aminolevulinate synthetase, the first enzyme located in mitochondria. We found that this enzyme could be induced by a variety of porphyrinogenic chemicals in rat and mouse livers and the mouse livers and the induction was blocked by the simultaneous administration of heme<sup>3,4</sup>. We proposed a model for the regulation of this enzyme based on heme acting as a negative effector molecule into an inactive one<sup>5</sup>.

### Heme and Biogenesis of Mitochondria

It is well known that the biogenesis of the mitochondrion involves coordination between the expression of the mitochondrial and nuclear genes. For the first time, we demonstrated in *Neurospora* that the heme deficient mitochondrion is defective in protein synthesis at the translation level<sup>6</sup>. We have also felt that heme may be the molecule that coordinates the expression of the nuclear and mitochondrial genes involved in the biogenesis of the mitochondrion. Towards this end, we have been looking at cytochrome c oxidase, which is considered

---

Professor, Department of Biochemistry, Indian Institute of Science, Bangalore-560012; Residence : E-18, New Quarter Complex, Indian Institute of Science, Bangalore-560012.

to be a mitochondrion in miniature. We studied the mechanism of translocation of the nuclear-coded subunits of cytochrome c oxidase into mitochondria and came up with the unexpected observation that these subunits are synthesized as precursors on the endoplasmic reticulum and translocation into mitochondria involves physical association of the endoplasmic reticulum with the mitochondrion<sup>7,8</sup>. This suggestion is not in tune with the accepted concept that the nuclear-coded mitochondrial proteins are synthesized only on membrane-free polyribosomes, released into the supernatant and then taken up by mitochondria. We are yet to study the role of heme in the translocation and assembly processes of oxidase.

In order to study the coordination at the gene level, we have constructed cDNA clones for the nuclear-coded subunits of cytochrome c oxidase<sup>9</sup>. Recently, we have isolated genomic clones from a  $\lambda$  library of rat liver DNA. We have yet reached a stage to study the role of heme in the coordination process.

### Heme and Cytochrome P-450 Gene Expression

Cytochrome P-450 is an excellent model system to study eukaryotic gene expression. Since this protein family is involved in drug metabolism and is also induced in an inducer-specific manner, it also affords an excellent opportunity to understand the gene basis for drug metabolism. We have constructed and characterized cDNA clones<sup>10</sup> and genomic clones for specific species of cytochrome P-450 induced by prototype drugs, such as phenobarbitone 3-methylcholanthrene. We have shown that the drugs induce the *de novo* synthesis of the specific species and

their messenger RNAs<sup>11,12</sup>. Using a variety of recombinant DNA techniques, we have established that these chemicals act at the level of transcription in activating specific cytochrome P-450 genes. We have also shown that inhibitors of heme synthesis block cytochrome P-450 gene transcription. Thus, the chemical and heme are both required for the transcription of the cytochrome P-450 genes<sup>10,13,14</sup>. Heme also influences the stability of the cytochrome P-450 protein. The apo-protein is unstable and degrades much faster than the holo-protein in the liver cell<sup>15</sup>.

One common thread running through the entire series of studies is the enquiry to understand the role of heme in living processes. With the introduction of cloning techniques, we are now in a definitive position to look for the interaction of heme with the regulatory sites of the gene, thus regulating gene expression. The other levels of heme action also need further analysis. The study with the cytochrome P-450 system has opened up the entire complex question of the gene basis of drug metabolism and brooks a rational answer to understand the ability of liver to handle such a wide variety of chemicals, which in complexity rates next only to the antigen-antibody system.

Earlier, I have also contributed to the understanding of the biochemical mode of action of  $\beta$ -N-oxalyl-L,  $\alpha$ ,  $\beta$ -diaminopropionic acid, the *L. sativus* neurotoxin.

### Selected Publications

1. Muthukrishanan S, Padmanaban G & Sarma P S, Regulation of heme biosynthesis in *Neurospora crassa*, *J biol Chem*, **224** (1969) 4241.



2. Jayaraman J, Padmanaban G, Malathi K & Sarma P S, Role of heme synthesis during mitochondriogenesis in yeast, *Biochem J*, **121** (1971) 531.
3. Rao M R S & Padmanaban G, Biochemical effects of the porphyrinogenic drug allyisopropylacetamide, *Biochem J*, **134** (1973) 859.
4. Rajamanickam C, Rao M R S & Padmanaban G, On the sequence of reactions leading to cytochrome P-450 synthesis. Effect of drugs, *J biol Chem*, **250** (1975) 2305.
5. Padmanaban G, Rao M R S & Malathi K, A model for the regulation of  $\delta$ -amino laevulenate synthetase in rat liver, *Biochem J*, **134** (1973) 869.
6. Chandra Kumar C & Padmanaban G, Role of heme in the synthesis of cytochrome oxidase in *Neurospora crassa*, *J biol Chem*, **225** (1980) 11130.
7. Parimoo S & Padmanaban G, Studies on the biosynthesis of the cytoplasmic subunits of cytochrome oxidase in rat liver, *Biochem biophys Res Commun*, **95** (1980) 1673.
8. Parimoo S, Rao N & Padmanaban G, Cytochrome c oxidase is preferentially synthesized in the rough endoplasmic reticulum-mitochondrion complex of rat liver, *Biochem J*, **268** (1982) 505.
9. Parimoo S, Seelan R S, Desai S, Buse G & Padmanaban G, Construction of a cDNA clone for a nuclear-coded subunit of cytochrome c oxidase from rat liver, *Biochem biophys Res Commun*, **118** (1984) 902.
10. Ravishankar H & Padmanaban G, Regulation of cytochrome P-450 gene expression—Studies with a cloned probe, *J biol Chem*, **260** (1985) 1588.
11. Bhat K S & Padmanaban G, Cytochrome P-450 synthesis *in vivo* and in a cell-free system from rat liver, *FEBS Lett*, **39** (1978) 337.
12. Bhat K S & Padmanaban G, Studies on the biosynthesis of cytochrome P-450 in rat liver—A probe with phenobarbital, *Archs Biochem Biophys*, **198** (1979) 110.
13. Ravishankar H & Padmanaban G, Effect of cobalt and 3-amino-1,2,4-triazole on cytochrome P-450 synthesis in rat liver, *Archs Biochem Biophys*, **225** (1983) 16.
14. Sathyabhama S & Padmanaban G, Effect of thioacetamide on cytochrome P-450 synthesis in rat liver, *Biochem J*, **213** (1984) 71.
15. Ravishankar H & Padmanaban G, Turnover of messenger RNA, apo-protein and haeme of cytochrome P-450 b + e induced by phenobarbitone in rat liver, *Biochem J*, **229** (1985) 73.

## G N Ramachandran

Ramachandran is one of the founders of the subject of molecular biophysics. In fact, he has established two flourishing schools in X-ray crystallography and molecular biophysics—the Molecular Biophysics Unit in the Indian Institute of Science, Bangalore and the Department of Crystallography and Biophysics in the University of Madras.

His early training was in the fields of optical diffraction theory, crystal physics, X-ray diffraction and crystallography, in Prof. C V Raman's laboratory in Bangalore and later in Prof. Bragg's laboratory in Cambridge, UK. On his return to Bangalore from Cambridge in 1949, he initiated crystal structure determination studies at the Indian Institute of Science. In 1952, he joined the University of Madras as Professor and Head of the newly started research Department of Physics of the University of Madras and this was the starting point of his serious studies on X-ray crystallography—both in its theoretical aspects and particularly in relation to structure determination of biomolecules. Along with (late) Gopinath Kartha, he worked out the triple helical structure of collagen which has been a starting point for the elucidation of chemical structure and biochemical properties of collagen in the decades thereafter. This was followed by a series of studies in X-ray crystallography using refined techniques of

Fourier transforms applied to anomalous dispersion data and the postulation of new types of syntheses related to structure analysis.

The studies on collagen led Ramachandran to the broader field of biomolecular structure and conformation, and a pioneering study in this field was the enunciation of the  $(\phi, \psi)$ -plot for a pair of peptide units in proteins and polypeptides, which is known now-a-days as the Ramachandran plot or diagram. This was followed soon after by a similar description of the essential backbone dihedral angle for polynucleotides and polysaccharides. During the 1960's, his laboratory in Madras was one of the leaders in the world working on all three types of biopolymers—proteins, nucleic acids and polysaccharides.

He moved over to Bangalore in 1970 where his colleagues Sasisekharan and V S R Rao also joined him in establishing the Molecular Biophysics Unit there, which has produced outstanding work in biopolymer research. In 1978, Ramachandran changed over his field to Mathematical Logic and has been engaged in studies on the computerization of logic using a unified Boolean vector-matrix formalism for the whole of logic, including propositional calculus, quantified predicate calculus and multivalued logic. Algorithms



have been prepared for the former two of these and he is actively pursuing this field as INSA Albert Einstein Professor.

Ramachandran's experience in the application of Fourier transforms in optics and X-ray crystallography led him to work out a new technique for the "reconstruction of substance from shadow" in X-ray tomography. This was the use of convolution integrals instead of Fourier transforms for image reconstruction—a technique which is now-a-days widely employed in CATSCAN equipment.

Being one of the earliest to develop the theory of biomolecular conformation, Ramachandran was invited to write a comprehensive review on the "Conformation of Proteins and Polypeptides" for the *Advances in Protein Chemistry*<sup>1</sup>. This review, written by him and Sasisekharan, is the most widely quoted article in this field (according to the Current Citation Index). So also, his paper on the quantum chemical theory of the non-planar peptide unit<sup>2</sup> is one of the most widely quoted references during the last decade, of a paper published by an author in the third world.

The sections that follow contain brief details of the studies conducted by Ramachandran and colleagues on collagen, conformation theory, X-ray crystallography and tomography, and mathematical logic. He has written several other reviews on these subjects, including one on crystal optics in the *Handbuch der Physik*, and special mention may be made of the book on *Fourier Methods in Crystallography*<sup>3</sup>, which presents a novel approach for this subject based on the studies made in Madras.

### (1) Triple Helical Structure of Collagen

In the early 1950's, several structures had been proposed for collagen and at the time when Ramachandran entered the field, this subject was wide open, and none of the structures proposed explained all the properties of collagen and the physico-chemical investigations made on it, such as X-ray diffraction, optical activity, infrared spectroscopy, etc. Dr Kartha and Ramachandran proposed the correct first approximation to the structure<sup>4</sup> in the form of three interrelated helices, each having three residues per turn, with a pitch of approximately 9Å, and crosslinked by NH...O hydrogen bonds. The most striking property of the structure was the occurrence of glycine at every third position in the amino acid sequence (demanded by the molecular packing in the structure), which fitted the one-third fraction of Gly residues in the amino acid composition. The structure also agreed with the diffraction pattern pretty well, the infrared dichroism, which required that NH and CO bonds were approximately perpendicular to the helical axis, and also the negative optical rotation of  $-300^\circ$  (which was demanded by the left-handed helices, which was essential in the structure if L-proline residues were to be incorporated). This was published in *Nature* in 1954. After a thorough re-examination of the X-ray pattern, a super-helical structure having 3.3 residues per turn was worked out<sup>5</sup>, which fitted the X-ray data very well. It provided the stimulus for various advances in connective tissue research, related to the physical chemistry and biochemistry of collagen. During the next 20 years, various improvements were made on the structure, the most important of which was the theoretical discovery<sup>6</sup>

that L-hydroxyproline occurring in the third residue of each three-residue bit takes part in a set of interconnecting hydrogen bonds via water molecules between neighbouring chains of the protofibril, which increases the stability of the triple helical structure. By this time, various consequences of the triple helical structure had been confirmed experimentally by different techniques and the higher stability imparted by hydroxyproline, as well as the occurrence of closely bound water molecules in the triple helical structure were also confirmed by experimental studies in other laboratories.

While editing his book on *Biochemistry of Collagen* mentioned below, Ramachandran had noticed that Complement C1<sub>q</sub> of the immune response system contains the sequence gly-X-Y for a good length of its chain structure, as in collagen, and has also many places where hydroxyproline occurs in the third position. While visiting Oxford on the way to the NIH in Bethesda, as a Fogarty Scholar, he learnt that the electron micrographs of complement indicated, as expected from theory, that it has a triple helical structure. During intensive discussions with scholars at NIH, he could obtain from this the remarkable conclusion that vitamin C is essential for the activity of the immune response system, as has been widely enunciated by Linus Pauling. The reasoning is as follows. It had been discovered during the previous decade that vitamin C is essential for the hydroxylation of proline in collagen. Since hydroxyproline is also essential for the stability of the triple helix in complement, it follows that vitamin C is fundamental for the synthesis of complement, a vital component of the immune response

system. These ideas were published by him in the Pauling Festschrift Number of the *International Journal of Quantum Chemistry, Quantum Biology Symposium*, in 1978<sup>7</sup>.

A comprehensive "*Treatise on Collagen*"<sup>8</sup> was published in 1967 (Academic Press), covering various aspects of the biochemistry and biology of collagen. In 1976, another book entitled "*Biochemistry of Collagen*"<sup>9</sup> (Plenum Press) was brought out which covered the advances made in this field mainly during the previous 10 years. Both were edited by Ramachandran and also contained individual chapters written by him.

## **(2) Studies on Conformation of Proteins and Other Biopolymers**

During the 1950's, when the theoretically postulated structure of collagen was improved from stereochemical criteria, one lacuna was found in the current state of the theory, namely, there were no clearcut conditions that had been postulated to distinguish between a "good" structure and a "bad" structure. Therefore, a thorough analysis of the published literature on biomolecules, such as amino acids, peptides, etc., was made and the particular segment of two peptide units meeting at a carbon  $\alpha$ -carbon atom was examined in detail from theory. It was then possible to show that the dihedral angles about N—C $^{\alpha}$  and C $^{\alpha}$ —C', termed now-a-days as ( $\phi$ ,  $\psi$ ), were the determining factors in defining the local conformation of a pair of peptide units. From a set of permissible contact distances arrived at by the analysis mentioned above, the range of values possible for ( $\phi$ ,  $\psi$ ) were worked out in full. (The investigation took over a year for conclusion.) When this was plotted in a map, with  $\phi$  and  $\psi$  as the X- and Y- axes, a



very neat diagram was obtained showing which regions of  $(\phi, \psi)$  are permissible and which are not<sup>10</sup>. The map completely fitted with all the available data in the literature and very soon thereafter  $(\phi, \psi)$  values for myoglobin (the first protein structure to be solved) were also found to fit the diagram very well.

These initial studies were followed up with a large number of investigations on various aspects of protein structure by Ramachandran and his colleagues—such as the chain building algorithm and techniques of energy minimization, the discovery of the stable conformation of three peptide units forming the  $\beta$ -bend (a standard feature in all protein structures), studies on helix formation and relative stabilities of various helices, analysis of all the amino acid side chain conformations and their incorporation in protein structure (in particular of proline), theory of the non-planarity of the peptide unit and its influence on peptide structures, study of the helices and conformations produced by alternating L and D peptide units (as they occur in peptide antibiotics), theory for the rare, but not impossible, occurrence of *cis* peptide units, and so on.

In view of the good success obtained for polypeptide chains, the same stereochemical approach was applied by Ramachandran and his colleagues in 1967 to polysaccharide and polynucleotide chains, and a set of five dihedral angles  $(\phi, \psi, \theta_1, \theta_2, \theta_3)$  were identified for describing the backbone conformation of the polynucleotide chain<sup>11</sup>, and similarly two dihedral angles about the bonds  $C_1-O$  and  $O-C'_4$  were identified for polysaccharide chains<sup>12</sup>. These have been followed up in great detail by the members of his group.

These ideas have become incorporated into the subject of biopolymer conformation, and they have been seminal for the explanation of biological activity in terms of structure and conformation of the constituent chemical molecules.

### 3. Crystallography

One of Ramachandran's early studies was the development of a technique for recording the X-ray topograph of diamond, namely, a picture showing the variation of perfection over the area of a plate of the crystal<sup>13</sup>. Published in 1944-46, these were probably the earliest application of X-ray diffraction for delineating the variation of perfection in crystals in a photographic manner. The name "topograph" for such pictures was coined by Professor Raman and adopted by Ramachandran in his papers.

During the decade 1949-59, he devoted his attention mainly to crystal structure analysis (both theory and practice) and the following may be mentioned in particular.

(a) Studies on crystal perfection and its variation by using polarized X-rays, along with the development of the dynamical theory, with special reference to X-ray "anti-reflection" (Renninger effect).

(b) Measurement of Bijvoet inequality and utilizing this for solving the "phase problem" in crystallography, a technique widely employed in the field now-a-days.

(c) Developing new techniques for Fourier syntheses employing partial information of phases. Of the three syntheses ( $\alpha$ ,  $\beta$  and  $\gamma$ ) that were developed, the  $\alpha$ -synthesis is equivalent to the technique employed for image reconstruction in holography.

#### 4. Three-dimensional Image Reconstruction

While he was in Chicago in 1970-71, he applied his knowledge of Fourier transform techniques in crystallography to the problem of image reconstruction in three dimensions making use of the data from two-dimensional projections, and a very efficient method of doing this (in which the Fourier reconstruction was converted into a convolution integral) was worked out<sup>14,15</sup>. This has later come to be designated as the convolution method, and is widely employed in CATSCAN instruments for radiology. This publication was the earliest report pointing out the application of the convolution method for three-dimensional image reconstruction from shadowgraphs

#### 5. Mathematical Philosophy

During the last eight years, Ramachandran has turned his attention to the development of a new formalism for the algebraic representation of logical expressions, in a form that is particularly suitable for direct computer implementation, in both software and hardware, in terms of electronic logic gates. The main achievements are the following:

(a) The development of a 4-valued logic (named Syad Nyaya System (SNS)<sup>16</sup> having the new states 'doubtful' (D) and 'impossible' (X), in addition to 'true' (T) and 'false' (F), which are expressed as 2-element Boolean vectors. Logical connectives are representable by  $2 \times 2$  Boolean matrices and the connective operation by Boolean matrix multiplication. These simple ideas are capable of faithfully implementing all logical operations required for sentential logic and are particularly fitted for computers. It has been extended to the theory of relations in

general, which is particularly applicable for data processing.

(b) The theory has been extended to quantifiers<sup>17</sup> such as "For all", "For some", which are representable by 3-element Boolean vectors and whose connectives are representable by  $3 \times 3$  Boolean matrices. This makes the implementation of predicate logic, and also higher order logic, capable of being carried out in terms of arithmetical operations, just as for sentential logic.

(c) Some of the circuits developed in connection with the above theory appear to have possibilities in the representation of logical relations in hardware<sup>18</sup> in computers.

This new approach of using the Boolean vector-matrix formalism for logic appears to have great possibilities both for providing an elementary treatment of some of the basic ideas and particularly for application in computer science.

#### Selection Publications

1. Ramachandran G N & Sasisekharan V, Conformation of polypeptides and proteins, in *Advances in protein chemistry*, Vol 23 (Academic Press) 1968, 283.
2. Ramachandran G N, Lakshminarayanan A V & Kolaskar A S, Theory of the non-planar peptide unit, *Biochim biophys Acta*, **303** (1973) 8.
3. Ramachandran G N & Srinivasan R, *Fourier methods in crystallography*, edited by M J Buerger (Wiley-Interscience, New York) 1970.
4. Ramachandran G N & Kartha G, Structure of collagen, *Nature, Lond*, **174** (1954) 269.
5. Ramachandran G N & Kartha G, Structure of collagen, *Nature, Lond*, **176** (1955) 593.
6. Ramachandran G N, Bansal Manju & Bhatnagar R S, A hypothesis on the role of hydroxyproline in stabilizing the collagen structure, *Biochim biophys Acta*, **322** (1973) 166.



7. Ramachandran G N, Confirmation of Pauling's theory that vitamin C improves immunity to infections, *Int J Quantum Chem, Quantum Biology Symposium*, **5** (1978) 15.
8. *Treatise on collagen*, edited by G N Ramachandran, Vol 1, *Chemistry of collagen* (Academic Press, London and New York) 1967.
9. *Biochemistry of collagen*, edited by G N Ramachandran and A H Reddi (Plenum Press, New York) 1976.
10. Ramachandran G N, Sasisekharan V & Ramakrishnan C, Stereochemistry of polypeptide chain configurations, *J molec Biol*, **7** (1963) 95.
11. Ramachandran G N, Sasisekharan V & Lakshminarayanan A V, Stereochemistry of nucleic acids and polynucleotides. I. Theoretical determination of the allowed conformations to the monomer unit, in *Conformation of biopolymers*, Vol 2, edited by G N Ramachandran (Academic Press, London), 1967, 641.
12. Ramachandran G N, Rao V S R, Sundarajan P R & Ramakrishnan C, Conformational studies of amylose, in *Conformation of biopolymers*, Vol 2 (Academic Press, London) 1967, 72.
13. Ramachandran G N, X-ray topographs of diamond, *Proc Indian Acad Sci*, **A19** (1944) 280.
14. Ramachandran G N & Lakshminarayanan A V, Three-dimensional reconstruction from radiographs and electron micrographs. II. Application of convolutions instead of Fourier transforms, *Proc natn Acad Sci, USA*, **68** (1971) 2236.
15. Ramachandran G N & Lakshminarayanan A V, Three-dimensional reconstruction from radiographs & electron micrographs : Part III—Description and application of the convolution method, *Indian J pure appl Phys*, **9** (1971) 997.
16. Ramachandran G N, Syad Nyaya System (SNS)—A new formulation of sentential logic and its isomorphism with Boolean Algebra of Genus 2, *Curr Sci*, **51** (1982) 625.
17. Ramachandran G N, Vector-matrix representation of Boolean Algebras and Application to Extended Predicate Logic (EPL), Parts I & II, *Curr Sci*, **52** (1983) 292, 335.
18. Ramachandran G N, New hardware circuits for the implementation of logical relations in information processing—Parts I II, *Curr Sci*, **55** (1986) 12, 67.

## L K Ramachandran

Proteins and peptides are of great biological importance. What are their structures? What is unique in each individual structure to account for its specific biological activity? These are the types of questions that have engaged the attention of Ramachandran for many years. The molecules that have been studied range from the simple amino acids and their derivatives, peptide antibiotics, enzymes and hormonal precursors, to toxins and viruses.

Original contributions from earlier years were wide ranging in nature. Proteins and amino acids in the thyroid gland and the nature of protein-iodine interactions had been investigated by Ramachandran in detail, prior to 1953, during his doctoral studies. The following three years saw him engaged in studies on proteins of wheat, chemical modification studies on gliadin, new isothiocyanates for structural investigations on proteins and peptides of the posterior pituitary gland. It is interesting to recall that in a publication on newer isothiocyanates, he had pointed out the suitability of a reagent like azobenzene isothiocyanate in the Edman degradation of peptides and commented that "modification of the reagent by the introduction of hydrophilic substituents could be expected to yield a reagent with better solubility properties, more suitable

in the structural investigation of peptides". The dimethylaminoazobenzene isothiocyanate, more popularly identified as DABITC, which was introduced by Chang in the seventies and was extensively used in manual micro-sequencing of proteins has now fulfilled that expectation. The period 1956 to 1959 witnessed Ramachandran's participation in a group effort at the Virus Laboratory of the University of California at Berkeley on elucidation of the structure of tobacco mosaic virus protein, then the third largest protein that was being tackled in the world for structure. There was extensive use of countercurrent distribution, a rare example, in separating the constituents of complex mixtures of peptides. In 1959, his investigations had laid the basis for oxidative modification of indole groupings in proteins with N-bromosuccinimide and selective cleavage of tryptophanyl peptide bonds in proteins. In the years that followed, he was engaged in the establishment of the structure of the linear peptide antibiotic gramicidin A, establishing the existence of a newer species by countercurrent distribution studies on commercial gramicidin samples, and identification and isolation of newer amino acids from species of plants belonging to the genus *Lathyrus*. That included L-homoarginine and  $\gamma$ -hydroxyhomoarginine. Procedures for the



isolation of lathyrine and its biosynthesis have been subjects of continuing interest to him. That mercuric acetate can cause acetoxymercuration of indole groupings in proteins with dramatic changes in ultraviolet absorption that may have analytical applications has been established.

### Peptide Antibiotics

Structure-activity correlations have been established for gramicidin A, which is a formyl pentadecapeptide ethanolamide with an alternating -L-D- amino acid sequence and three -(D-Leu-Trp)- repeats. The four indole groupings of the four tryptophan residues were found to play an equal and important role in conferring antibacterial activity on the molecule. The primary hydroxyl group of the ethanolamide moiety has been clearly implicated in so far as the haemolytic activity and toxicity of the antibiotic are concerned. This knowledge has enabled the preparation of O-methyl or O-carboxymethyl or des-ethanolamine gramicidins, which possess little to none of the haemolytic activity or toxicity, but retain in full measure the antibacterial activity of gramicidin A. These derivatives are, therefore, more appropriate for potential therapeutic use. The above knowledge has permitted laboratory synthesis of antibacterially active synthetic peptides retaining the essential region of the gramicidin molecule. These include di-, tetra-, octa- and deca-peptides containing repeating sequences of -(D-Leu-Trp)- or -(D-Leu-Phe)- or -(D-Leu-Trp)- with the termini protected to different extents, and protected and unprotected octapeptides having the same sequence as gramicidins A, B and C between residue locations 8 to 15. Some of the data would seem to favour the later evolutionary origin of gramicidin

A in *Bacillus brevis* which also produces minor quantities of gramicidins B and C containing phenylalanine or tyrosine in lieu of the tryptophan residue found at location 11 in gramicidin A. This study has yielded nontoxic derivatives of gramicidin and many antibacterially active synthetic peptides.

Structure-activity correlation studies have been made on polymyxin B, which finds more therapeutic use than gramicidin. Formylation and separation have yielded mono-, di-, tetra- and penta-formyl polymyxins of which all but the last two are even more active than polymyxin, pointing to the essentiality of no more than two of the five amino groups of side chains of the five diaminobutyric acid residues present in the molecule. This is in line with another observation that polymyxin and its mono-, di- and tri-formyl derivatives form nearly the same depth of colour in the Folin and biuret reactions, whereas the tetra- and penta-formyl derivatives will yield only a fifth as much colour. Also, none of the polymyxin derivatives or polymyxin will form colour in the Folin reaction in the absence of  $\text{Cu}^{2+}$  ions. In examining the copper complexing ability using a modified Job method, the first group of the three biologically active derivatives will form under conditions of copper excess 1 : 3 complexes, whereas the two inactive derivatives will form only 1 : 1 complexes. There is reason to believe that the two additional complexing sites, two amino groups, in the derivatives which are active, relative to those that are not, are involved in the biological action of polymyxin B, which may be mediated through interactions with cations. The two sites (amino groups) are contributed by residues 1 and 3. In the diformyl derivative, the essential amino groups are those of

residues 1, 3 and 5. The role of these amino groups in the sequestration of biologically important cations, such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  from the outer layer of cell membranes of sensitive bacteria, is now being examined.

In therapeutics, combination antibiotics have a certain importance because of the existence of exploitable additive, synergistic and potentiation effects. While many combinations are still in use, none involving only peptide antibiotics is known. We have discovered a unique mutual potentiating effect in the antibiotic actions of linear gramicidin and polymyxin B. These two antibiotics are without effect on gram-negative and gram-positive organisms, respectively. It has been found that gramicidin potentiates polymyxin's activity on gram-negative organisms to the extent of 2- to 13-fold. If in *in vitro* assays inoculum cultures are pretreated with the potentiating antibiotic, the enhancements observed are 6- to 32-fold. Evidence showing the binding of the potentiating antibiotic by the test organisms is available. What is unique about this effect is that none of the many other peptide antibiotic combinations involving polymyxin B and others, such as gramicidin S, thiostrepton, etamycin and rifomycin, displays such effects. Additionally, the potentiations observed are found to be valid *in vivo* in experimental infections (*S. typhosa*, *A. aerogenes*) in mice in which 4- to 6-fold enhancements in the biological activity of polymyxin B by linear gramicidin can be observed. This makes us feel that the polymyxin B-linear gramicidin combination may be used in infections more profitably, with the advantage of lowered dosage. This approach seems appealing, as derivatives of gramicidin with little

haemolytic activity and toxicity are now available and some infections are intractable with more conventional therapy.

### Toxic Amino Acids

Seeds of *Lathyrus sativus* which is grown extensively in certain parts of India form part of the dietary of people in such areas and are held responsible for the crippling disease lathyrism. In earlier studies by other workers, one toxic amino acid, namely  $\beta$ -N-oxalyldiaminopropionic acid, had been recognized. Our studies have shown that N,N'-dioxalyldiaminopropionic acid also is present to the extent of 0.3%, that it too is toxic (lethal) to several types of experimental animals and that it adversely affects levels of glutaminase and acetylcholinesterase of brain and liver of animals. A good analytical method for the separation of oxalic acid and the two oxalyl derivatives of diaminopropionic acid has been developed. The toxicity of the seeds is believed to arise because of the above three compounds.

### Buffalo Thyroglobulin

The endocrinology of the (Indian) buffalo is a neglected area. It has been noted that thyroids of animals of the Hyderabad geographic region (elevation 2000 ft) contain very little iodine (0.003-0.01%) compared to those of the plains (Madras; Egypt). The importance of thyroid status in relation to productivity of farm animals is known. In private, it has been made known to us that Hindustan Lever Company had after our study undertaken iodine supplementation for its herds in the uplands and observed 10% increase in milk production. From further studies of the thyroid it has been shown that purified thyroglobulin of the buffalo is



a glycoprotein of molecular weight 700,000 and it shares with proteins from other species many compositional and other characteristics. One of these is extreme heterogeneity. However, the protein appears to be unique in regard to its amino terminus.

### Chemical Modification of Proteins

The use of acetic anhydride-formic acid as a reagent for formylation of amino and hydroxyl groups of proteins and the rates at which these reactions occur in model proteins like lysozyme and papain have been investigated thoroughly. The selective removal of O-formyl groups at alkaline pHs leaving behind only N-formyl groups has been accomplished with the two proteins. The influence of such masking on the biological activity of the two proteins and the ability to regenerate the native proteins, with biological activity intact, by solvolytic removal of the N-formyl groups, has been demonstrated. The formyl group can be used for the reversible masking of aliphatic hydroxyl and amino groups of proteins. Further, during these studies, an old method for determining N-acetyl groups as N-2,4-dinitrophenyl-N-acetylhydrazine has been refined based on data on the differing susceptibilities of acetamido and formamido groups to hydrazinolysis to yield a very sensitive and reliable colorimetric micromethod for the determination of formyl groups as N-formyl-N'-2,4-dinitrophenylhydrazine.

### Toxins of Animal Venoms

A chromatographic method for large scale separation of constituents of the venom of the Indian cobra (*Naja naja*) has been developed. Two neurotoxins and two

cardiotoxins have been characterized in terms of amino acid composition. Structure work, by degradation and by fast atom bombardment mass spectrometry (in collaboration), on the cardiotoxins has been initiated. Cardiotoxin II has been shown to possess pyrophosphatase activity too. It has been characterized as a metalloprotein, containing magnesium, which requires metal for its enzymatic activity but not for its cardiotoxicity. The toxin displays a new type of membrane activity. The lysis of bacteria which can be induced by lysozyme is prevented by it. The reason for this lies in not antagonizing cell wall hydrolysis by lysozyme, which proceeds normally, but in interactions with the inner membrane of bacteria to confer stability against osmotic lysis to the protoplasts. The protection afforded by this agent is unrivalled, and such protoplasts are as sturdy as bacteria with intact cell walls. Magnesium ions at optimal moderately high concentrations can destabilize such protoplasts.

Toxins of scorpions with effects on blood pressure and on smooth muscle and which have post- or pre-synaptic actions on the nervous system are also being investigated in association with pharmacologists.

Ramachandran intends to devote his energies, as he does at the moment, to enlarge activities and understanding in the areas of manual microsequencing of protein structures, synthesis of biologically active peptides, the biosynthesis of lathyrine and the complex peptide antibiotic thiostrepton, and structure-function relations and the mode of action of cardiotoxins.

## Selected Publications

1. Ramachandran L K, Protein-iodine interaction, *Chem Rev*, **56** (1956) 199-218.
2. Ramachandran L K & McConnell W B, 3-o-Nitrophenyl- and 3-phenyl-azophenyl-2-thiohydantoins of amino acids, *J Am chem Soc*, **78** (1956) 1255-57.
3. Gish D T, Ramachandran L K & Stanley W M, Studies on the amino acid sequence of tobacco mosaic virus protein. I. Fractionation of products of tryptic hydrolysis by countercurrent distribution, *Archs Biochem Biophys*, **78** (1958) 433-50.
4. Fraenkel-Conrat H & Ramachandran L K, Aspects of the structure of tobacco mosaic virus, in *Advances in protein chemistry*, Vol 14 (Academic Press, New York) 1959, 175-225.
5. Ramachandran L K, Biological applications of infrared spectroscopy, in *Infrared spectroscopy* by C N R Rao (Academic Press, New York) 1963, 479-513.
6. Ramachandran L K & Witkop B, Selective cleavage of C-tryptophyl bonds in peptides and proteins, *J Am chem Soc*, **81** (1959) 4028-32.
7. Ramachandran L K, The use of N-bromosuccinimide in the study of the composition and structure of proteins, *J scient ind Res*, **21C** (1962) 111-27.
8. Ramachandran L K, Morphology and structure of plant viruses, *Proc natn Inst Sci India*, **24** (1963) 115-28.
9. Ramachandran L K, On the heterogeneity of gramicidin, *Biochemistry*, **2** (1963) 1138-42.
10. Ram S L N, Ramachandran L K & Adiga P R, The isolation and characterization of L-homoarginine from seeds of *Lathyrus sativus*, *Biochemistry*, **2** (1963) 298-300.
11. Ramachandran L K & Witkop B, N B S cleavage of peptides, in *Meth Enzymol*, **11** (1967) 283-99.
12. Witkop B, Ishii S, Sarges R, Sakiyama F, Ramachandran L K & Gross E, Aminosäuresequenz des gramicidins A, *Angew Chem*, **76** (1964) 793.
13. Ramachandran L K, The gramicidins, *J scient ind Res*, **34** (1975) 249-65.
14. Ramachandran L K, Nitrogenous constituents of lathyrus plants and the problems of lathyrism, in *Chemistry of natural products-three decades* (Andhra Pradesh Academy of Sciences, Hyderabad) 1975, 183-92.
15. Srinivasa B R & Ramachandran L K, The polymyxins, *J scient ind Res*, **38** (1979) 695-709.
16. Ramachandran L K, Srinivasa B R & Radhakrishna G, The structural requirements for the biological activity of polymyxin B, in *Peptide antibiotics*, edited by H Kleinkauf and H von Dohren (Walter de Gruyter, Inc, Berlin) 1982, 428-43.
17. Ramachandran L K, Achyuthan K E, Agarwal O P, Chaudhury L, Vedasiromani R & Ganguly D K, Toxic proteins of snakes and scorpions, *Proc Indian Acad Sci (Chem Sci)*, **93** (1984) 1117-36.
18. Ramachandran L K, *A foundation course in science and technology*, edited by L K Ramachandran (Andhra Pradesh Open University, Hyderabad) 1983, 1984, pp 235.



## N Ramanathan\*

The research work done by Ramanathan was in the nature of a vital link between fundamental research on collagen, such as molecular structure, on the one hand, and applied and developmental work on leather, on the other. It is a bridge between the science and technology of collagen and has been unique and outstanding, representing a systematic application, for the first time, of physical and biophysical techniques to problems of leather and footwear research. This has necessarily involved an extensive study of the physical and mechanical properties and the electron microscopical structure of collagen fibres, which are the major constituents of hides and skins and leather, and of how these are modified by various chemical treatments, diseases, heat, ultraviolet, gamma and neutron radiations, source of collagen and age of the animal from which the collagen fibres are obtained.

In normal collagen fibrils, a number of cross-striations are seen under the electron microscope. When these are treated with vegetable tanning materials like myrobalan or wattle, the cross-striations are not seen and the fibrils appear coated. Some tanning materials like chrome or formaldehyde do not cause any coating, while some others like fish oil and sulphonyl chloride coat the fibrils only

partially. This observation gives a method of classifying tanning materials based on the electron microscopical appearance of the collagen fibrils; this method is useful for predicting the behaviour of new tanning materials towards collagen fibres. The mechanical properties of collagen fibres yielding coated fibrils are different from those of fibres yielding uncoated or partially coated fibrils. The tensile strength or torsional rigidity of the fibres depends on the additional crosslinks formed by the treatment and also on the physical deposits in the form of coatings. While the crosslinks influence the inherent mechanical properties, the coatings influence the extent of cohesion between the fibrils, which, in turn, influences these properties.

It was discovered that collagen fibres exhibit what is known as the directional friction effect (DFE). The coefficient of static friction in one direction of the fibre axis is greater than that in the opposite direction. Woollen garments shrink when washed or muddled, because this difference in the coefficients of friction forces the wool fibres to migrate and the shrinkage is prevented by nullifying DFE by suitable treatments. The discovery of DFE in collagen fibres has led to a method of correcting what is called the "looseness of the grain" in heavy leathers. While

---

Emeritus Scientist and Formerly, Director, Central Leather Research Institute, Adyar, Madras-600020;  
Residence : 17, Third Cross Road, Karpagam Gardens, Adyar, Madras-600020.

\*Since deceased

collagen fibres are held firmly in the corium, they are somewhat loose in the region between the corium and the grain layers, especially in hides which are very thick or heavy. In processing, these loose fibres are subject to the same type of forces as the wool fibres in woollen garments and they migrate, tending to form "knots". This results in weak adhesion between the grain and the corium, giving rise to the loose grain effect. Ramanathan showed by electron microscopy that DFE in collagen fibres is due to the saw-toothed appearance of the surface of the fibres and that any treatment by which the fibrils lose their cross-striations nullifies DFE, as saw teeth appear only with cross-striations. In the particular case of the loose grain effect in heavy hides, correction of the defect could be achieved by treating the collagen fibres (i.e. the hides) with polymers which could cause a coating on the fibre surface. The discovery of DFE is also useful in preparing collagen felts. The effects of tannages, other chemical treatments, age of the animal from which the collagen fibres were obtained, the source of collagen and ultraviolet and gamma radiations on DFE of collagen fibres, were also investigated.

The correlation thus established between the electron microscopical structure of the fibrils and the mechanical and frictional properties of collagen fibres, which, in turn, decide, for the most part, the properties of hides, skins and leathers, is a unique contribution.

Diseases of the skins result in transverse breakages of the collagen fibrils, as seen in the electron microscope; such breakages were established to be the cause of the decrease in the tensile strength of the collagen fibres from the diseased portions

of skins. Ultraviolet, neutron and gamma radiations have deleterious effects on leather and collagen fibres at dosages higher than 2.5 mrad, soluble and insoluble collagen being affected to different extents. Vegetable tanning materials prevent deterioration in the properties of collagen fibres and leather better than other tanning materials.

Collagen fibres were found to have optimum properties at the "prime of life" of the animal. The physical properties and electron microscopical appearance were found to vary with the source of collagen and its location on the animal influences the composition and properties of skins and the resultant leathers.

Removal of non-collagenous materials decreased the tensile strength and the tensile modulus and increased the torsional rigidity of collagen fibres. Collagen fibres treated with vegetable tanning materials after removal of the non-collagenous materials exhibited a higher tensile strength than those treated without removal of these materials. These results are useful for preparing leathers with improved properties. The pore sizes in collagen were also determined and this helped in understanding the mechanism of tannages, as the site at which tanning materials get located or react in the collagen fibres could be identified.

It was demonstrated that during hydrothermal shrinkage of collagen fibres, hides, skins or leathers, a close correlation exists between the behaviour of the fibrils, as seen in the electron microscope, and that of the collagen matrix in the untanned or tanned states. It was shown that the hydrothermal stability of leather, even at the fibril stage, is not uniform, as the same fibril remains intact in some others. This



ties up with the observations made by other workers that the chemical structure or the physical coherence may vary over the same fibril.

Compressibility measurements showed that the higher the soluble content in collagen, the softer is the tissue. This finding is of help in preparing collagen articles with desired properties.

A structure was suggested for elastoidin to explain the 3-band pattern seen under the electron microscope.

The physical and chemical characteristics of dugong hide collagen were studied to show how environment may influence such characteristics.

In the field of footwear, a "comfort index" was arrived at based on extensive investigations on the temperature and relative humidity inside shoes while being worn. The shoes were fabricated using a variety of leathers and/or synthetic materials. The experiments were conducted under moderate and extreme climatic conditions to assess the suitability of various types of materials in different climates. The comfort index gives an indication of the extent of comfort a pair of shoes might impart to the wearer. Electromyographic and electrogoniometric studies were carried out to establish the action of the particular muscles which results in the bending of the foot at the ankle and toe joints and also to determine the angles through which such flexion takes place with and without footwear. The results facilitate the design and choice of materials to be used in footwear for ensuring comfort for normal and abnormal feet.

The discovery was made that the ratio of the length to the width of a foot is a

constant within narrow limits. Based on this, it was suggested that the width interval could be 2 mm between consecutive sizes and that for each length, there should be 5 fittings (5 width values) so that the users of footwear will have a better choice, as two persons may have feet of the same length but the width of their feet may be different within the narrow limits stated above. The shoes fabricated according to the new system proved to be better than those made according to the prevalent system when tests were made on children and adults. It was also observed that the feet of children grow in such a manner that the ratio of length to width tends to remain constant at the value observed for a normal foot.

Future researches would be in the direction of more practical applications of the knowledge gained on the structure and properties of collagen fibres and their interrelationship for purposes like the development of sheet materials from leather wastes, of newer leathers with desired properties and of comfortable footwear for abnormal and afflicted feet.

### Selected Publications

1. Lokanadam B, Usha R, Venkatappaiah B & Ramanathan N, Electron microscopical studies on the structure of ultrasonically treated collagen fibres in relation to their tensile properties, *Proc Third Asia-Pacific Conference on Electron Microscopy*, Singapore, 1984, 439.
2. Rajaram A & Ramanathan N, Regenerated collagen monofilaments with improved tensile strength, *Proc First International Symposium on Bioengineering*, Calcutta, 1983, 83.
3. Rao D L V, Venkatappaiah B, Shanmugam I S & Ramanathan N, Electromyographic and electrogoniometric studies of dorsiflexors, *Proc Fourth annual conference of the American Society of Biomechanics*, Vermont, USA, 1980.
4. Rajaram A, Sanjeevi R & Ramanathan N, The mechanical properties of tanned collagen fibres, *Leath Sci*, **25** (1978) 419.

5. Sanjeevi R, Viswanathan B & Ramanathan N, Pore size distribution in collagen fibres using water vapour adsorption studies, *Colloid Interface Sci*, **57**(2) (1976) 207.
6. Sanjeevi R & Ramanathan N, Role of the non-collagenous components in the physical properties of tanned collagen fibres, *Leath Sci*, **23** (1976) 1.
7. Venkatappaiah B & Ramanathan N, A study on the rate of growth of the feet of children, *Leath Sci*, **19** (1972) 358.
8. Nayudamma Y, Kamat D H, Muthiah P L & Ramanathan N, Studies on shoe climate contributing to comfort, *J Soc Leath Tr Chem*, **54** (1970) 226.
9. Krishna Sen & Ramanathan N, A study of collagen fibres from goat skins infected with *Demox caprae*, *Leath Sci*, **17** (1970) 269.
10. Ramanathan N, The effect of age on the biophysical characteristics of collagen fibres, *Q J surg Sci*, **4** (1968) 7.
11. Mohanaradhakrishnan V & Ramanathan N, Studies on elastoidin. Part II—An electron microscope study, *Leath Sci*, **13** (1966) 137.
12. Goverdhan Rao D P & Ramanathan N, Friction of collagen fibres, *J Am Leath Chem Ass*, **60** (1965) 704.
13. Mohanaradhakrishnan V, Nayudamma Y & Ramanathan N, Effect of gamma radiation on soluble and insoluble collagens, *Biochim biophys Acta*, **102** (1965) 533.
14. Thomas Joseph K, Nayudamma Y, Rajendran K R & Ramanathan N, Studies on Dugong hide collagen, *J Am Leath Chem Ass*, **59** (1964) 462.
15. *Collagen*, edited by N Ramanathan (John Wiley, New York) 1962.



## V S R Rao

Until recently, carbohydrates had received much less attention compared to the other two biopolymers, namely, nucleic acids and proteins. This has been due, in part, to the extreme complexity of carbohydrates which are endowed with several degrees of conformational freedom. Conformational studies on them in solution and in solid state have been hampered on account of their poor solubility and the difficulty in obtaining well-oriented fibres. Also, the importance of carbohydrates in multifarious biological processes has begun to be appreciated only recently. In the face of difficulties in studying these molecules using experimental techniques, theoretical methods were developed by Rao for investigating their structure and conformation. These studies have, over the years, been expanded to encompass carbohydrate conjugates and carbohydrate-protein interactions.

### Conformation of Polysaccharides and Sugars

An early application of theoretical methods led to the successful prediction of the conformation of amylose<sup>1</sup> in the solid state and in solution. It has been shown that all the sugar residues in amylose exist only in chair conformation and that it forms a left-handed helix. These predictions were later confirmed by X-ray studies. The stereochemical features

worked out for a number of other polysaccharides have revealed definite correlations between their shapes and biological functions<sup>2-4</sup>. The methods developed for the study of random polysaccharide chains, besides giving valuable information about their dimensions and probable shape in solution, have also led to an understanding of the puzzling problem that existed in polymer chemistry, namely, the reason for the existence of the extended conformation of cellulose in contrast to the compact conformation of amylose<sup>4,5</sup>.

The preferred conformations of a number of simple sugars have been worked out theoretically<sup>6,7</sup>. These studies have explained many of the observations of Reeves on the sugar ring and have provided a firm theoretical basis for the possible distortions in the pyranose ring, the effect of substituents and the anomeric effect on the stability of chair conformations.

Recent molecular orbital calculations of Rao and coworkers have given a clear understanding of the nature of the electronic interactions that take place in anomeric systems which contain adjacent polar bonds. Also studied are the electronic interactions that occur in other biological molecules like the phosphates of nucleic acids and carbohydrates, and the sulphates of heparin<sup>8</sup>. MO calculations on

the above and other systems containing multiadjacent polar bonds have led to the predictions of preferred conformations, and of the dependence of bond lengths and bond angles on the torsion angles. Such structural features have also been investigated in phosphomethanediol, which is a model compound for glucose-1-phosphate in which two multipolar bonded systems are combined.

### $\beta$ -Lactam Antibiotics

The bacterial cell wall (peptidoglycan) is an important carbohydrate conjugate and penicillin is a lactam antibiotic that inhibits its growth. The mode of action of this antibiotic has been investigated using stereochemical criteria<sup>9</sup>. This study revealed that penicillin blocks the crosslinking reaction in peptidoglycan biosynthesis by assuming conformations that are similar to the X-D-Ala-D-Ala portion of the peptide segment. The high potency of penicillin is due to its more precise fit for the enzyme than the substrate itself. This model also provided information on the important roles of the lactam peptide bond, the conformation of the bicyclic ring system, and the nature and orientation of 'R' groups, which are important for the design of new drugs active against penicillin resistant strains.

The geometry optimization of the  $\beta$ -lactam structure, using MO calculations, has demonstrated that the peptide bond length is a function of the hybridization state of nitrogen and also of conjugation with the second ring in the antibiotic molecule. The MO study has also led to the correlation of the biological activity of  $\beta$ -lactam antibiotics with the optimized geometry of the  $\beta$ -lactam structure.

### Blood Group Substances

The blood group substances are glucoproteins, which are present in secretions. They carry antigens similar to the ABH(O) and Lewis antigens present on the red blood cell of an individual. These antigens are complex carbohydrates, the chemical structures of which are known. The possible conformations of the A, B, H(O), Le<sup>a</sup> and Le<sup>b</sup> oligosaccharides have been computed theoretically. It has been shown that the conformation of the common core fragment of these oligosaccharides is not altered significantly on the addition of the monosaccharide residues, L-fucose, D-galactose, and N-acetyl-D-glucosamine, at the nonreducing end, which gives the various blood group specific antigens. Using the most probable conformations arrived at from these calculations, correlations have been made between the shapes of those antigens and their interactions with enzymes, antibodies and lectins<sup>10</sup>. It has been suggested that the blood group specific reagents can be classified into two groups: those which cannot distinguish between type 1 and type 2 structures and those which are specific either for a type 1 or a type 2 structure. If the reagent is in the first group, it should have a small binding site. Those in the second group may have either a large binding site or, if the binding site is small, they may be sensitive to local changes in the structure of the antigen in the neighbourhood of the major determinant sugar residue. In the case of the blood group oligosaccharides, the most important local change responsible for differences in type 1 and type 2 structures is the interchange of the N-acetamide and hydroxymethyl group on the N-acetyl-D-glucosamine residue.



## Gangliosides

Gangliosides are glycolipids which occur mainly in the plasma membrane of nerve cells. They are characterized by the presence of negatively charged sialic acid residues. The oligosaccharide moieties are possible receptors for cholera toxin, tetanus toxin and sendai virus. The possible conformations of these gangliosides which have been well characterized as far as their chemical structure is concerned have been computed theoretically<sup>11</sup>. An attempt has also been made to correlate their shapes to their interactions with the toxins, the virus and some neuraminidase enzymes. It has been suggested that cholera toxin has a small binding site while for tetanus toxin and sendai virus the binding sites may be large accommodating more than two sugar residues. The conformational similarity between the sialic acid (2→3) galactose fragment of GM3 and GDIA and the sialic acid (2→8) sialic acid fragment in some of the other gangliosides has thrown some light on the specificity of some of the neuraminidase enzymes.

## Carbohydrate-Protein Interactions

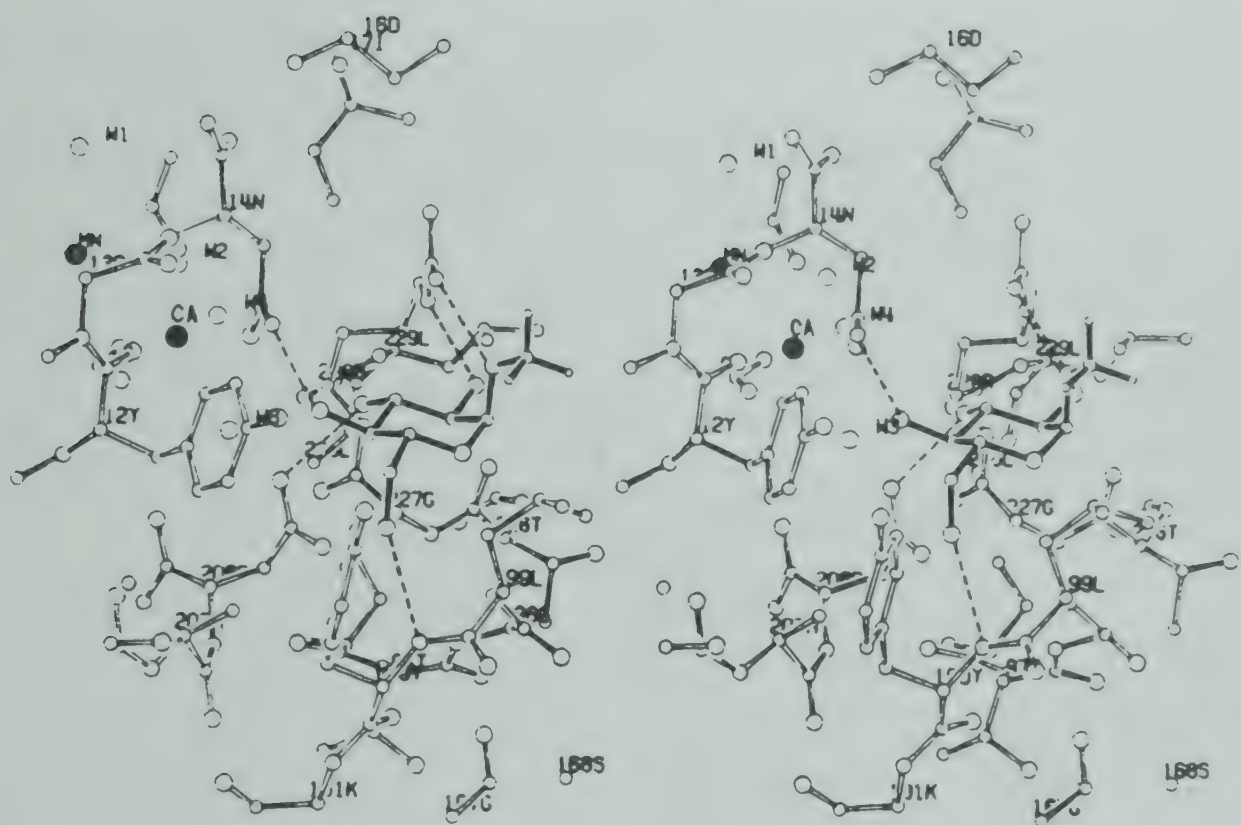
Earlier attempts made to correlate the shapes of complex oligosaccharides with their biological functions have led to a more intensive study of protein-ligand interactions through the development of computer experiments.

The modes of binding of various sugars to Concanavalin A determined by computer modelling experiments<sup>12,13</sup> led to predictions about the effect of the nature of the substituent and its orientation at different carbon atoms in the pyranose ring, on its binding modes and affinity to

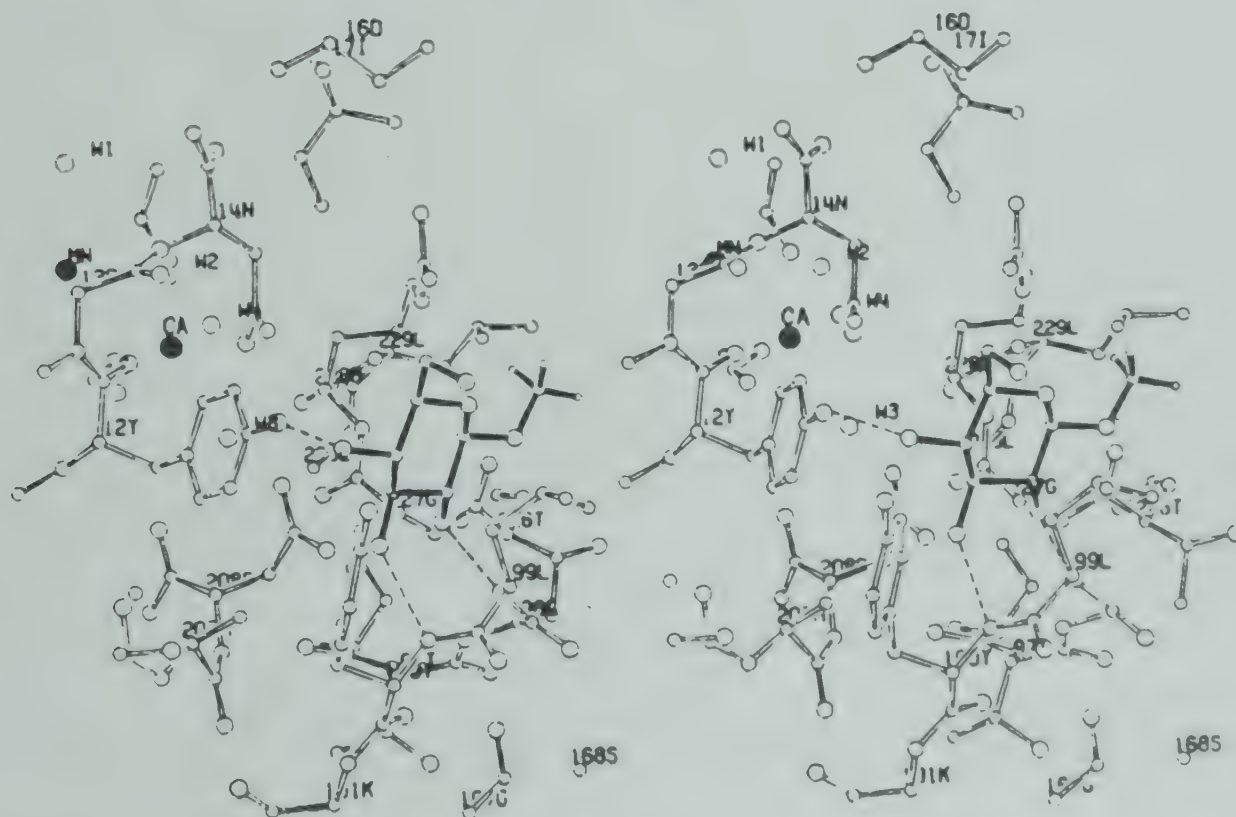
Con A. Methyl  $\beta$ -D-glucopyranoside and methyl  $\alpha$ -( $\beta$ )-D-mannopyranoside can bind to Con A in three different modes, whereas methyl  $\alpha$ -D-glucopyranoside can bind only in one mode. Methyl- $\beta$ -D-glucopyranoside in its most favourable mode of binding differs from methyl  $\alpha$ -D-glucopyranoside in its alignment in the binding site of Con A where it binds in a flipped or inverted orientation (Fig. 1). In the extended binding site, such a flipping in the orientation of the pyranose ring glycosidically linked to the sugar unit placed in the primary binding site is also observed in some oligosaccharides. This affects the direction of binding of oligosaccharides, and the preference of a particular residue to reach the binding site. These studies also suggest that molecular fit and the non-covalent forces are important in the binding of carbohydrates to lectins.

## Flavodoxin-FMN Binding

Flavodoxins are low molecular weight FMN-containing proteins which function as electron carriers in a variety of microbial metabolic processes. Earlier, it had been generally assumed that conformational changes occur in the apoflavodoxin during the binding process but not in FMN. However, the computer modelling studies<sup>14</sup> carried out clearly indicate that the conformers of FMN that initiate the binding process undergo significant changes in the position of the phosphate group, to reach that final bound conformation. In the final bound conformation, the phosphate group forms a network of hydrogen bonds with apoflavodoxin and contributes significantly to the binding energy to offset the electrostatic repulsions from Met 56 and Glu 59. This explains the importance of



(a)



(b)

Fig. 1. Projection of (a) Con A-methyl- $\alpha$ -D-glucopyranoside complex; and (b) Con A-methyl- $\beta$ -D-glucopyranoside complex.



the phosphate group in the binding of FMN to apoflavodoxin.

### Thermolysin-inhibitor Binding

The studies carried out on thermolysin with different inhibitors<sup>15</sup> have indicated that small changes in the structure of the inhibitor may lead to flipping of the inhibitor in the active site. A comparison of the modes of binding of a number of inhibitors suggests that changes in configuration or the size of the inhibitor affect their alignment in the active site. The initial requirement for binding seems to be that the inhibitor in one of its preferred conformations should have a partial or full complementarity to the active site. The former will lead to an induced fit binding, whereas the latter leads to a simple lock-key fit. In general, a large number of attachments to the active site and a tight fit are important for binding.

### Future Plan

Drug design and interaction of biomolecules involved in recognition and other biological processes is proposed to be studied through computer modelling experiments.

### Selected Publications

1. Rao V S R, Sundararajan P R, Ramakrishnan G & Ramachandran G N, *Conformation of biopolymers*, Vol II, edited by G N Ramachandran (Academic Press, London) 1967, 721.
2. Sathyanarayana B K & Rao V S R, Conformational studies of  $\beta$ -D-(1,3) linked xylan, *Carbohyd Res*, **15** (1970) 147.
3. Rao V S R, Conformational analysis of polysaccharides, in *Structural aspects of biomolecules*, edited by R Srinivasan (Macmillan India Ltd) 1981.
4. Sundararajan P R, Yathindra N & Rao V S R, Configurational statistics of polysaccharide chains. Part I. Amylose, *Biopolymers*, **8** (1969) 325.
5. Yathindra N & Rao V S R, Configurational statistics of polysaccharide chains. Part II. Xylan and mannan, *Biopolymers*, **10** (1971) 1605.
6. Sundararajan P R, Vijayalakshmi K S & Rao V S R, Theoretical studies on the conformation of aldohexopyranoses, *Carbohyd Res*, **17** (1971) 341.
7. Joshi N V & Rao V S R, Studies on the conformational flexibility in the pyranose ring in  $\alpha$ - and  $\beta$ -D-glucose, *Biopolymers*, **18** (1979) 2993.
8. Saraswathi V & Rao V S R, Theoretical investigations on the structure of  $\text{H}_2\text{SO}_4$ ,  $(\text{CH}_3\text{O})_2\text{SO}_2$  and  $\text{CH}_3\text{OSO}_3$  Kallanan, *J molec Struct (Theo Chem)*, **92** (1983) 19.
9. Rao V S R & Vasudevan T K, Conformation and activity of  $\beta$ -lactam antibiotics, *Crit Rev Biochem*, **14** (1983) 173.
10. Rao V S R & Biswas Margaret, Conformation and interactions of blood group carbohydrates, in *Topics in carbohydrates*, edited by E D T Atkins (Mac Millan Publishers Ltd, UK) in press.
11. Veluraja K & Rao V S R, Theoretical studies on the conformations of higher gangliosides, *Carbohyd Polymers*, **3** (1984) 315.
12. Chandrasekharudu Y & Rao V S R, The modes of binding of methyl  $\alpha$ -(and  $\beta$ ) D-glucopyranoses and some of their derivatives to concanavalin A, *J Biomol Str Dyns*, **2** (1984) 41.
13. Chandrasekharudu Y & Rao V S R, Theoretical studies on the modes of binding of some of the derivatives of D-mannose to concanavalin A, *Int J Biol Macromolec*, **6** (1984) 337.
14. Vinayaka C R & Rao V S R, A conformation approach to study the modes of binding of flavin mononucleotide to flavodoxin, *J Biol Str Dyns*, **2** (1984) 663.
15. Ghosh Indira & Rao V S R, A conformational approach to the study of the dynamics of enzyme inhibition studies on thermolysin, *Int J Biol Macromolec*, **4** (1982) 130.

## V Sasisekharan

Sasisekharan has been engaged in research on the structure of compounds of biological interest for more than two decades. The main lines of investigations pursued by him concern the structures of proteins and nucleic acids and he has published more than 100 research papers in these fields in leading international journals.

The proteins are the chief building materials of living systems and the nucleic acids are the carriers of genetic information. It is well-known that the complicated functions of these macromolecules are intimately connected with their structure, for all their properties follow from their structure.

The detailed molecular structure of collagen, the protein of the connective tissue, was worked out in Madras and Sasisekharan was an active member of the group responsible for the solution of the structure of collagen. The molecule of collagen is a triple helical structure and this was investigated thoroughly by Dr G N Ramachandran and Sasisekharan (1965). These studies have now become a textbook matter for students of structural biology. As an adjunct to the studies on collagen, physico-chemical and X-ray studies on poly-L-proline and poly-L-hydroxyproline which are related to collagen were made. He worked out the

crystal structures of these polypeptides and that perhaps was the first structural study made on these polypeptides. In addition, he initiated work on the theoretical determination of the possible conformations of a polypeptide chain based on stereochemistry; this work has been recognized as the first significant step in our understanding of folding of peptide chains. Later, this was followed by extensive studies on the effect of the side chains on the backbone conformations and vice-versa in a polypeptide chain from energy considerations. He also initiated at Madras work on the conformations of biological molecules from quantum mechanical considerations. A comprehensive review on the conformation of polypeptides and proteins was written by him and Prof. G N Ramachandran.

Subsequent to his joining the Indian Institute of Science, Bangalore, he initiated structural work on DNA. Natural DNA fibres are usually dimorphic. The dimorphic forms, A and B, are interconvertible in the solid phase such that a change of handedness of the duplex is not possible under the conditions of transition. Thus, it was conjectured that both forms were necessarily right-handed, since earlier attempts to build left-handed double-helical structures for A- or B-DNA met with little success. On the other hand,



for the first time it was recognized by us that an enormous degree of conformational flexibility is inherent in the nucleotide unit, the building block of the polynucleotide duplex, and at the junction of two neighbouring nucleotides in polymers. This flexibility was made use of extensively in our building of molecular models, studies that were done following stereochemical guidelines. Thus, it was possible not only to identify the stereochemical nature of this flexibility, but also to show that both right- and left-handed structures of DNA, some of which were subsequently seen in single crystals, followed as a natural outcome of the flexibility.

Fibre diffraction data (resolution of  $\sim 3$  Å) defy any direct determination of the structure of DNA in contrast to single crystal analysis at high resolution ( $\sim 1$  Å), which allows determination of the molecular details of the structure. Even after almost three decades following the proposal of the double helix, only a few structures of oligomeric DNA have been resolved atomically. The paucity of such structural data called for an altogether independent and complementary approach in the study of the plausible conformational variants of DNA, which we undertook almost a decade ago. The approach involved model building studies through stereochemical considerations.

The flexibility of the furanose ring plays a pivotal role in linking bases with the backbone in a polynucleotide chain. Earlier model-building studies retained a rigid geometry for the sugar, since the nature of the flexibility of the furanose ring was not available then. Furthermore, the geometry of the nucleotide unit was not fully understood and the model-building studies

of polynucleotides proceeded simultaneously with the determination of the crystal structures of nucleotides. Even when the refinement of the polynucleotide structures was made through X-ray fibre diffraction data by Arnott and coworkers in 1970, only information from crystal structures of nucleotides was available. However, such data provide information only about the stereochemistry of nucleotides and not the relative orientation of the nucleotides around the 3-5 phosphodiester bonds. This relative orientation is the key factor that determines the secondary structure of nucleic acids, and details of this orientation can be obtained from single crystal studies of dinucleotide monophosphates and higher oligomers. Only during the last few years a few crystal structures, mostly of dinucleoside monophosphates and a few higher oligomers, have become available for correlation and comparison. Systematic analyses were made of the then available crystallographic data mostly on dinucleoside monophosphates and a couple of higher oligomers and their features obtained. These data revealed certain correlations among the major torsional degrees of freedom present in the structure and these were used as a stereochemical guideline for model building purposes.

Initially, using stereochemical guidelines, double helices with mononucleotides as the repeating units were investigated by us. This led to the elucidation of left-handed DNA duplexes, hitherto ignored, and at the same time significantly improved upon the understanding of the existing right-handed models. These duplexes were compatible with the fibre diffraction data of various forms of DNA

and did not permit discrimination between right- and left-handed models.

Subsequently, molecular conformations of DNA with alternating purine and pyrimidine sequences were studied. It was observed that there could be a variety of sequence-specific and conformationally distinct right-handed and left-handed duplexes.

The possibility of DNA in either handedness raised the question whether one could join a segment of a right-handed structure with a segment of a left-handed structure. It was indeed shown to be possible due to the conformational flexibility of the sugar phosphate backbone. The link possessed the allowed stereochemistry of the backbone and retained classic Watson-Crick base pairing. Bases, however, had a novel kind of stacking arrangement called the inverted stacking which was shown to be energetically as favourable and also as frequently observed in single crystals as the normal type. The joining of the right- and left-handed double-helical fragments resulted in a new structure of DNA called the RL model. Thus, for the B-DNA, a structure with alternating right-helical and left-helical segments of approximately 5 bp in a repeat of 10 bp was proposed. Further details and implications of this structure and a comparison with the double helix are given elsewhere.

Interestingly, the first ever solved single crystal structure of short length of DNA double helix turned out to be left-handed. Alex Rich and coworkers solved the crystal structure of a hexa-nucleotide (CGCGCG) having alternating G and C bases. Though the base pairing scheme is of the Watson-Crick type, the phosphates of the polynucleotide backbone trace a zig-

zag left-handed roughly helical path and hence the structure was termed as Z-DNA. Again, the next crystal structure, a tetranucleotide with alternating purine pyrimidine sequences (CGCG), solved by Dickerson and coworkers, turned out to be similar and left-handed. Both these discoveries were of interest to us, as our earlier investigations had indicated that the exact topology of the polynucleotide chains would be a function of the preferred conformations of the nucleotides of the purine and pyrimidine bases and these reports confirmed this.

Subsequently, in 1980, Dickerson and coworkers observed a right-handed B-DNA-like conformation in the crystal structure of the duplex of a dodecamer, CGCGAATTCGCG. It was observed that there could be conformational variants within B-DNA itself and the structure of the dodecamer is sequence specific. Thus, in 1979-1980, the existence of both right- and left-handed structures in the fragments of duplex DNA was established in crystals.

Thus, it is clear that the structure of DNA is not entirely made up of right-handed double helix only, as suggested by Watson and Crick. For the many functions that DNA has to perform, it is obvious that duplex DNA should have a variable structure along its length. From what has been discussed above, it is clear that both right- and left-handed duplexes are plausible. It is, therefore, very likely that segments of right- and left-handed duplex would be present, giving rise to RL segment in DNA structure, since a stable link can be made between them. Two questions that arise then are: What are the maximum lengths of continuous right- and left-handed segments of DNA, and under what conditions would these occur?



### Selected Publications

1. Ramachandran G N & Sasisekharan V, Cylindrical lattice structure of collagen, *Archs Biochem Biophys*, **63** (1957) 255.
2. Sasisekharan V, Structure of poly L-proline II, *Acta Cryst*, **12** (1959) 897.
3. Ramachandran G N & Sasisekharan V, Structure of collagen, *Nature, Lond*, **190** (1961) 1004.
4. Sasisekharan V, Stereochemical criteria for polypeptide and protein structure, in *Collagen*, edited by N Ramanathan (Wiley Publishers, New York) 1962, 39.
5. Ramachandran G N, Ramakrishnan C & Sasisekharan V, Stereochemistry of polypeptide chain configurations, *J molec Biol*, **7** (1963) 95.
6. Sasisekharan V, Conformation of polynucleotides, in *Proc Jerusalem Symposia on Quantum Chemistry and Biochemistry*, Vol 5, edited by E D Bergmann and B Pullman (Academic Press, New York) 1973, 247.
7. Sasisekharan V & Pattabiraman N, Structure of DNA predicted from stereochemistry of nucleoside derivatives, *Nature, Lond*, **275** (1978) 159.
8. Gupta Goutam, Bansal Manju & Sasisekharan V, Conformational flexibility of DNA: Polymorphism and handedness, *Proc natn Acad Sci*, **77** (1980) 6486.
9. Gupta Goutam, Bansal Manju & Sasisekharan V, Polymorphism and conformational flexibility of DNA: Right and left handed duplexes, *Int J Biol Macromolec*, **2** (1980) 368.
10. Sasisekharan V, Bansal M & Gupta G, Structure of DNA re-examined, in *Structural aspects of recognition and assembly in biological macromolecules*, edited by M Balaban *et al.* (Academic Press, Jerusalem) 1980, 501.
11. Sasisekharan V, Gupta G & Bansal M, Sequence dependent molecular conformation of polynucleotides: Right- and left-handed helices, *Int J Biol Macromolec*, **3** (1981) 2.
12. Sasisekharan V & Pattabiraman N, Double stranded polynucleotides: Two typical alternative conformations for nucleic acids, *Curr Sci*, **45** (1976) 779.
13. Gupta Goutam, Bansal Manju & Sasisekharan V, Reversal of handedness in DNA: A stable link between RU and LZ helices, *Biochem biophys Res Commun*, **97** (1980) 1258.
14. Sasisekharan V, Pattabiraman N & Gupta G, Some implications of an alternative structure for DNA, *Proc natn Acad Sci, USA*, **75** (1978) 4092.
15. Sasisekharan V, Left-handed DNA duplexes, *Proc Cold Spring Harbor Symp quant Biol*, **47** (1983) 45.

## P S Sastry

It is a little over 30 years ago since I began my research career and all along my scientific investigations were confined mainly to 'lipids and biomembranes'—an area in which great progress has been made during the last three decades. Within this area, however, I drifted considerably and my investigations concerned a variety of lipids from vitamin A to glycolipids; diverse organisms—animals, plants and bacteria, and a number of animal tissues from the liver to the brain. During the early years, I worked with Prof. J Ganguly at Bangalore, with Prof. M Kates at Ottawa, Canada and with Prof. L E Hokin at Madison, Wisconsin (USA) and in later years a number of doctoral students were associated with me in my scientific endeavours. This fruitful association with many has much to do with my research accomplishments.

During the late fifties, we observed that vitamin A is absorbed and stored in the liver as its long-chain fatty acylester, the retinol palmitate<sup>1</sup> and established that a specific vitamin A esterase present in liver is responsible for the maintenance of blood levels of retinol<sup>2</sup>. We noted that the sodium salt of retinoic acid is biologically more active than all-*trans*-vitamin A<sup>3</sup>, indicating that the acid form is closer to the systemically active physiological form. Since then I have become interested in plant lipids, particularly the chloroplast

membrane lipids and their biosynthesis. We isolated the major lipid of the chloroplast membranes and established its structure as monogalactosyl diglyceride. This was soon followed by our discovery of a new enzyme, 'galactolipase', in runner bean leaves; these findings stimulated a great deal of interest in this class of lipids<sup>4</sup>. Isolation of a 'cerebroside' from photosynthetic tissues and establishment of the biosynthetic pathways for phospholipids in leaves<sup>5</sup> were the other accomplishments of that period.

Among bacteria, the halophilic bacteria are unique in their ability to survive in high salt media. We were the first to investigate the lipid components of this bacterium and we discovered that this organism contains 2,3-di-O-dihydrophytanyl glycerol-1-phosphoryl-glycerol-3-phosphate as the major lipid, an entirely new diether glycerolipid<sup>6</sup>. Since then the halophiles became a subject of intense study because of their purple membrane and the associated ability to convert light energy directly into chemical energy, i.e. ATP.

The role of lipids and proteins in membrane function is a fascinating subject to study. We investigated the metabolism of phospholipids during 'phagocytosis' by polymorphonuclear leukocytes<sup>7</sup> and found an active turnover of the acidic phospholipids, the phosphatidic acid and

---

Professor, Department of Biochemistry, Indian Institute of Science, Bangalore-560012; Residence : E-34, Staff Quarters, Indian Institute of Science, Bangalore-560012.



phosphatidyl inositol, an effect more commonly known as the 'phospholipid effect', which has since been recognized as one of the mechanisms involved in signal transduction. We, for the first time, showed that the  $\text{Na}^+$ ,  $\text{K}^+$  ATPase of brain (the enzyme responsible for the maintenance of ionic balance in all cells) is phosphorylated on the acyl group of the glutamic or aspartic acid residues, when ATP is hydrolysed<sup>8</sup>. It was since shown that the aspartyl residue is phosphorylated in many transport ATP-ases.

In the recent past, our research efforts have been concentrated on the biochemistry of developing brain and on the mechanisms of oil synthesis in the developing groundnut seeds. It is believed that undernutrition during the vulnerable period of growth impairs brain maturation and might lead to an irreversible deficit in higher mental function—a subject of great concern in our national context. We found that undernutrition, especially protein malnutrition, during the weanling period in rats, leads to a reduction in isolable myelin<sup>9</sup> and also impairs synaptogenesis in the brainstem<sup>10</sup>. To be able to extrapolate this finding to humans, we initiated a programme on the ontogeny of various neurotransmitter receptors in developing human foetal brains. We discovered that the muscarinic cholinergic receptors necessary for many motor functions are formed mainly during the third trimester of pregnancy<sup>11</sup>. This period is also important in the development of dopaminergic receptors. Myelination is one of the most important events that occur during brain development and ethanolamine plasmalogens are the major and characteristic lipids of myelin membrane. We established the biosynthetic pathway of this lipid in developing brain<sup>12</sup> and in this

context discovered an active fatty acyl CoA reductase<sup>13</sup> and a new cholesterol esterifying enzyme<sup>14</sup> in the developing brain. An equally important aspect is the initiation and regulation of synthesis of myelin membrane proteins which we are currently studying.

Seed oils are the main dietary sources of fats. However, the regulatory mechanisms involved in the channeling of photosynthetase to triglycerides in the developing oilseeds are not known. This information has potential application in enhancing oil biosynthesis. Therefore, we conducted experiments on developing groundnut seeds and discovered that these seeds use an alternative pathway for the formation of glycerol backbone and its phosphorylation<sup>15</sup>. In this pathway, glyceraldehyde-3-phosphate rather than dihydroxyacetone phosphate serves as the source for the 3-carbon fragment. Glyceraldehyde-3-phosphate is converted to glyceraldehyde by a specific phosphatase, which, in turn, is reduced to glycerol by an NADPH specific reductase. The glycerol thus formed is phosphorylated to glycerol-3-phosphate by a glycerokinase, which exhibits allosteric properties. The glycerophosphate is then acylated to give the first lipid biosynthetic intermediate, viz. phosphatidic acid. It was also observed that all the enzymes in this pathway are coordinately induced during the active phase of triglyceride synthesis. The possible genetic or hormonal control of these enzymes will now be investigated. The biosynthesis of sulfoquinovosyl diglyceride, a unique sulfolipid present in the chloroplast membranes, and the toxic effects of various chemical classes of herbicides on lipid metabolism in plants, are the other aspects currently under investigation in our laboratory.

## Selected Publications

1. Subba Rao K, Sastry P S & Ganguly J, Fatty acid component of vitamin A ester in sheep liver, *Archs Biochem Biophys*, **95** (1961) 285.
2. Sastry P S & Ganguly J, Studies on vitamin A esterase, *Biochem J*, **80** (1961) 397.
3. Malathi P, Subba Rao K, Sastry P S & Ganguly J, The biological activity of vitamin A acid in rats, *Biochem J*, **87** (1963) 305.
4. Sastry P S, Glycosyl glycerides, *Adv Lipid Res*, **12** (1974) 251.
5. Sastry P S & Kates M, Biosynthesis of lipids in plants—Incorporation of glycerophosphate- $P^{32}$  into phosphatides by cell-free preparations from spinach leaves, *Can J Biochem*, **44** (1966) 459.
6. Kates M, Sastry P S & Yengoyan L, Isolation and characterization of a diether analog of phosphatidyl glycerophosphate from *Halobacterium cutirubrum*, *Biochim biophys Acta*, **70** (1963) 705.
7. Sastry P S & Hokin L E, Studies on the role of phospholipids in phagocytosis, *J biol Chem*, **241** (1966) 3354.
8. Hokin L E, Sastry P S, Galsworthy P R & Yoda A, Evidence that a phosphorylated intermediate in a brain transport adenosine triphosphatase is an acyl phosphate, *Proc natn Acad Sci (USA)*, **54** (1965) 177.
9. Reddy P V, Das A & Sastry P S, Quantitative and qualitative changes in the myelin of undernourished and protein malnourished rat brains, *Brain Res*, **161** (1979) 227.
10. Reddy P V & Sastry P S, Studies on neurotransmitter-stimulated phospholipid metabolism with cerebral tissue suspensions: A possible biochemical correlate of synaptogenesis in normal and undernourished rats, *Brain Res*, **168** (1979) 287.
11. Ravi Kumar B V & Sastry P S, Muscarinic cholinergic receptors in human foetal brain: Characterization and ontogeny of ( $^3H$ )-quinuclidinyl benzilate binding sites in frontal cortex, *J Neurochem*, **44** (1985) 240.
12. Natarajan V & Sastry P S, Studies on the biosynthesis of ether-linked ethanolamine phospholipids in developing rat brain, *Indian J Biochem Biophys*, **12** (1975) 340.
13. Natarajan V & Sastry P S, Conversion of 1- $^{14}C$ -palmitic acid to 1- $^{14}C$ -hexadecanol by developing rat brain, *J Neurochem*, **26** (1976) 107.
14. Jagannatha H M & Sastry P S, Cholesterol-esterifying enzymes in developing rat brain, *J Neurochem*, **36** (1981) 1352.
15. Ghosh S & Sastry P S, Triacyl glycerol synthesis in developing seeds of groundnut (*Arachis hypogaea*), *Biochem J* (1986), communicated.



## C SivaRaman

### Molecular Enzymology

SivaRaman and his colleagues have done pioneering work on the purification and characterization of the citrate lyase complex (EC 4.1.3.6), an enzyme which initiates the anaerobic utilization of citrate in a number of bacteria by catalyzing the cleavage of the substrate to oxaloacetate and acetate in the presence of  $Mg^{2+}$ . Besides its importance in bacterial intermediary metabolism, the enzyme represents probably the first of the citrate enzymes to evolve.

Bacterial citrate lyase was obtained pure for the first time by SivaRaman<sup>1</sup> and this initiated extensive studies on the enzyme from *Klebsiella aerogenes*, the source from which it had been purified. The enzyme was shown to have a molecular weight of 575,000 and to dissociate in the presence of *p*-hydroxymercuribenzoate and in divalent metal-free buffers of low ionic strength<sup>2</sup>. Later studies carried out by other workers have shown that *K. aerogenes* citrate lyase is a multienzyme complex built up from six copies each of three different polypeptide chains of 54,000 ( $\alpha$ ), 32,000 ( $\beta$ ), and 10,000 ( $\gamma$ ) daltons, the  $\alpha$  and  $\beta$  subunits being enzymes with acyl-transferase and acyl-lyase activities, respectively, and the  $\gamma$  subunit with a covalently bound S-acetylated coenzyme A-like prosthetic group functioning as an

acylcarrier protein (ACP). The cleavage of citrate is mediated by the ACP subunit or by acetyl-coenzyme A when the ACP subunit is deacetylated. The overall reaction involves a sequence of two steps with the formation first of (3S)-citryl-ACP or (3S)-citryl-coenzyme A as an intermediate. The subsequent step is the cleavage reaction which has an absolute requirement for divalent metal ions, such as  $Mg^{2+}$ ,  $Zn^{2+}$  or  $Mn^{2+}$ . Divalent metal ion mediated modulation in the conformation of the enzyme complex from *K. aerogenes* has been shown by SivaRaman's group in equilibrium dialysis experiments using  $Mn^{2+}$  as a probe<sup>3</sup>, and as suggested by them this may represent one of the mechanisms by which the enzyme activity is regulated *in vivo*.

Citrate lyase from *K. aerogenes* undergoes rapid auto-inactivation through the deacetylation of its ACP subunit, the *in vitro* half-life time  $t_{1/2}$  value being about 30s. In marked contrast, the enzyme from *Streptococcus diacetilactis* had been shown to be stabler, having a  $t_{1/2}$  value of about 10 min. It was believed at the time that the stability was conferred by an associated acetylating activity present in a subunit of the *S. diacetilactis* enzyme complex. This was, however, shown to be incorrect through the isolation by SivaRaman's group of the *Streptococcus faecalis* enzyme which resembles the *S.*

---

Consultant, Division of Biochemical Sciences, National Chemical Laboratory, Pune-411008; Residence : E-55, NCL Colony, Pune-411008.

*diacetilactis* enzyme in its inactivation behaviour while carrying no associated acetylating activity<sup>4</sup>. The acetylating activity in the *S. diacetilactis* enzyme preparation was later found to be due to an impurity.

Studies on the active sites of citrate lyase had also been initiated for the first time by SivaRaman's group. The involvement of an essential arginine residue at each of the active sites of the acyl-transferase and acyl-lyase subunits of the *K. aerogenes* enzyme was shown from chemical modification studies<sup>5</sup>. The arginine residue of the transferase subunit was shown to be located at the citrate-binding site and the reactivity of this residue was shown to be greatly enhanced by the presence of acetyl-coenzyme A, substrate which exchanges its acetyl group with the citryl moiety in the course of the reaction. The enhanced accessibility of the active site residue reflects a modulation in the conformation of the acyl-transferase subunit in the presence of acetyl-coenzyme A. This behaviour has been explained on the basis of "alligator-type" model in which the subunit is assumed to be present normally in a closed form, but undergoes a conformational change to an open form when acetyl-coenzyme A occupies its binding site<sup>6</sup>.

*p*-Azidobenzoyl-coenzyme A has been used by SivaRaman's group as a photoaffinity reagent for specifically labelling the active site of the acyl-lyase subunits of the *K. aerogenes* citrate lyase complex<sup>7</sup>. The stoichiometry of the coenzyme A ester binding investigated by the use of *p*-azido[<sup>14</sup>C] benzoyl-coenzyme A indicated that all six acyl-lyase subunits in the complex are accessible to free coenzyme A mediated reaction, indicating

for the first time that the cleavage of citrate *in vivo* proceeds via both the built-in prosthetic group in the complex and through acetyl-coenzyme A. This also explains the function of the subunit combinations in the citrate lyase complex which are devoid of prosthetic groups.

The ACP subunit of *K. aerogenes* citrate lyase contains an S-acetylated cysteine residue in addition to the S-acetyl moiety on the coenzyme A-like prosthetic group. This had posed the problem of the biologically active acyl-carrier in the ACP subunit. Direct evidence for the cysteamine moiety of the prosthetic group being the active acyl-carrier was obtained by SivaRaman and his colleagues when they showed that the reaction-inactivation of citrate lyase results in deacetylation of the S-acetyl cysteamine residue, but not of the S-acetylated cysteine in the ACP polypeptide chain<sup>8</sup>. Evidence was also obtained for the spatial proximity of the cysteamine and cysteine residues in the ACP subunit, which permits intrapeptide disulphide bridge formation on mild oxidation of the deacetylated subunit. The involvement of the citrate lyase ligase which acetylates the prosthetic group in the post-translational modification of the cysteine residue in the ACP subunits was also established.

One of the most significant contributions made by SivaRaman's group is the purification and characterization of the citrate lyase complex from *Escherichia coli*<sup>9</sup>. The enzyme from this source has been shown to diverge from other citrate lyases in its subunits structure, containing a single large ACP ( $\gamma$ ) subunit of 85,000 daltons, which interacts with six copies each of acyl-transferase ( $\alpha$ ) and acyl-lyase ( $\beta$ ) subunits of the usual sizes. The



unusually large, fused ACP subunit of *E. coli* citrate lyase which carries four covalently bound pantothenate-containing prosthetic groups has a repetitive structure, particularly in the domains where the four prosthetic groups are located<sup>10</sup>. These structural features reflect internal gene duplications.

### Enzyme Engineering

A process for the immobilization of penicillin acylase (EC 3.5.1.11) was developed by the research group led by SivaRaman at NCL. The process was scaled up in collaboration with Hindustan Antibiotics Ltd (HAL). The immobilized enzyme preparation now finds industrial application at HAL for the manufacture of 6-aminopenicillanic acid, the key intermediate for the production of semisynthetic penicillins. This is the first reported industrial application of an immobilized enzyme in the country.

### Selected Publications

1. SivaRaman C, Purification of citrase from *Aerobacter aerogenes*, *Biochim biophys Acta*, **52** (1961) 212-13.

2. Mahadik S P & SivaRaman C, Citrate lyase: Molecular weight and subunit structure, *Biochem biophys Res Commun*, **32** (1968) 167-72.
3. SivaRaman H & SivaRaman C, Cooperative binding of manganese to citrate lyase from *K. aerogenes*, *FEBS Lett*, **105** (1979) 267-70.
4. Hiremath S T, Paranjpe S & SivaRaman C, Purification and properties of citrate lyase from *Streptococcus faecalis*, *Biochem biophys Res Commun*, **72** (1976) 1122-28.
5. Subramanian S, Basu A & SivaRaman C, The presence of essential arginine residues at the active sites of citrate lyase complex from *Klebsiella aerogenes*, *Biochem biophys Res Commun*, **111** (1983) 490-97.
6. Subramanian S & SivaRaman C, Bacterial citrate lyase, *J Biosci*, **6** (1984) 379-401.
7. Basu A, Subramanian S & SivaRaman C, Photoaffinity labeling of *Klebsiella aerogenes* citrate lyase by *p*-azidobenzoyl coenzyme A, *Biochemistry*, **21** (1982) 4434-37.
8. Basu A, Subramanian S, Hiremath L & SivaRaman C, S-Acylated residues of the acyl-carrier protein subunit of *Klebsiella aerogenes* citrate lyase, *Biochem biophys Res Commun*, **114** (1983) 310-17.
9. Nilekani S & SivaRaman C, Purification and properties of citrate lyase from *Escherichia coli*, *Biochemistry*, **22** (1983) 4657-63.
10. Subramanian S, Basu A, Nilekani S & SivaRaman C, Evidence for repetitive structure of the large acyl-carrier protein subunit of *Escherichia coli* citrate lyase, *Biochem biophys Res Commun*, **122** (1984) 1253-59.

## A Sreenivasan

Sreenivasan's research contributions, spanning a period of over 50 years, relate to biochemistry, nutrition and food science and food technology, with more than 200 publications authored by him and his coworkers.

### Metabolic Events and Radiation Effects

The areas of biochemical research in cell function and metabolism have related to (i) basic events and regulatory processes at the molecular level; (ii) function and structure of sub-cellular components; (iii) tissue differentiation and dedifferentiation processes; and (iv) elucidation of loci of molecular lesions in radiation injury.

### Food Science and Technology

Major areas of research pertain to (i) evaluation of the effects of processing procedures and unit operations on nutritional quality of food products; (ii) fermentative production of enzymes such as amylases, proteases, pectinases, etc. and of amino acids lysine and glutamic acid; (iii) extension in shelf-life of perishable foods by storage in controlled atmospheres, such as skin coating in fruits and refrigeration; (iv) disinfection of stored grains; and (v) packaging aspects of processed foods.

Important contributions on the development and clinical evaluation of low-cost protein-rich foods for combating protein deficiency states in pre-school and school children have shown the scope for (a) use of defatted oilseed meals like groundnut, cottonseed, sesame, coconut, etc. and, in a few cases, their protein isolates; and (b) production of a concentrated, bland fish flour from indigenously available and commercially unimportant fish varieties.

### Food Irradiation

Sreenivasan was responsible for initiating and developing several areas of research on low-dose radiation preservation of foods. These have included (a) disinfection of stored foodgrains; (b) sprout inhibition of potatoes and onions during storage; and (c) shelf-life extension of fruits, fish and other perishable foods. These studies have been extended, establishing the wholesomeness for human consumption of irradiated foods which are at least as nutritive as foods thermally or otherwise processed by the conventional methods.

### Selected Publications

1. Williams J N (Jr) & Sreenivasan A, Co-factor requirements of the tyrosine oxidase system of rat liver, *J Biol Chem*, **203** (1953) 605, 613; **205** (1953) 109.

---

Formerly, Head, Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Bombay 400085 and Research Director, Cancer Research Institute, Tata Memorial Centre, Bombay 400012; Residence : 132, 6A Cross & 9th Main Road, Raj Mahal Vilas Extension, Bangalore 560080.



2. Rege D V & Sreenivasan A, Conversion of oracil to thymine in strains of *Bacillus subtilis*, *J biol Chem*, **208** (1954) 471.
3. Fatterpaker P, Marfatia U & Sreenivasan A, Relationship between folic acid and nicotinamide metabolism, *Biochem J*, **59** (1955) 470.
4. Fatterpaker P, Marfatia U & Sreenivasan A, Role of folic acid and vitamin B12 in trans-methylations, *Indian J med Res*, **43** (1955) 43, 337, 343, 349.
5. Alimchandani H R & Sreenivasan A, Inhibition steps in sulfonamide bacteriostasis, *J Bact*, **73** (1957) 538; **74** (1958) 171, 175.
6. Mulgaonkar A G & Sreenivasan A, Vitamin B12 binding by rat serum protein fractions, *Archs Biochem Biophys*, **80** (1959) 22.
7. Fatterparker P, Manjrekar S P, Marfatia U, Mulgaonkar G, Rege D V & Sreenivasan A, Influence of vitamin B12 and folic acid on protein utilization in the growing rat, *J Nutr*, **71** (1960) 371.
8. Aiyar A S & Sreenivasan A, Co-enzyme Q metabolism in pantothenic acid deficiency, *Proc Soc exp Biol Med*, **107** (1961) 911.
9. Aiyar A S & Sreenivasan A, Sub-cellular distribution and biosynthesis of ubiquinone in the rat in experimental thyrotoxicosis, *Biochem J*, **82** (1962) 179, 182.
10. Katyare S S, Fatterpaker P & Sreenivasan A, Heterogeneity of rat liver mitochondrial fractions and the effects of tri-iodo-thyronine on their protein turnover, *Biochem J*, **118** (1970) 111.
11. Subba Rao M N, Netrawali M S, Pradhan D S & Sreenivasan A, Template activity of liver chromatin in rats following whole-body irradiation, *Indian J Biochem Biophys*, **8** (1971) 257.
12. Pradhan D S & Sreenivasan A, Basic mechanisms of radiation injury and repair in mammalian systems, *J scient ind Res*, **30** (1971) 703.
13. Rajwade M S, Katyare S S, Fatterpaker P & Sreenivasan A, Regulation of mitochondrial protein turnover by thyroid hormones, *Biochem J*, **152** (1975) 379.
14. Sreenivasan A, Perspectives in cancer research, *Nuclear India*, **14**(4) (1985) 4.
15. Sreenivasan A, Development and evaluation of processed foods based on peanut flour and its proteins, in *Protein needs of infants and children*, Publ No 843, US National Academy of Sciences, Washington, 1961, 227.
16. Subrahmanyam V, Sreenivasan A & Swaminathan M, Food needs in relation to nutritional requirements in *Proc Symposium on Food Needs and Resources* (Indian National Science Academy, New Delhi) 1962, 5, 134.
17. Kumta U S & Sreenivasan A, Food irradiation researches and pilot plant facilities in India, in *Proc FAO/IAEA International Symposium on Food Irradiation* (1966) 785.
18. Gore M S, Sawant P L, Kumta U S & Sreenivasan A, Dehydroirradiation process for shrimp, *Food Technol*, **24** (1970) 1164.
19. Vakil U S, Sreenivasan A, et al., Wholesomeness testing of irradiated foods, *Bhabha Atomic Research Centre Reports*, No 455 (1970); No 712 (1973); *Proc IAEA Symposium on Radiation Preservation of Foods*, 1973, 673.

## R Srinivasan

Srinivasan's basic degree was in physics, with specialization in X-ray crystallography, although later his interest shifted to biological structures and wide-range NMR applications. He has made substantial contributions in the following areas: (1) Theoretical and statistical aspects of crystallography; (2) X-ray structure analysis of small organic and biomolecules; (3) Biomacromolecules: Studies on protein folding; and (4) Wide-range NMR-structural applications.

### **(1) Theoretical and Statistical Aspects of Crystallography**

Much of the early work during the late fifties and sixties pertains to this category and was done largely in collaboration with Prof. G N Ramachandran and Dr S Parthasarathy. Development of Fourier techniques for structure analysis in a systematic fashion and application to phase problems under a variety of situations, such as when part of the structure is known and when anomalous scattering is present, etc., was the major objective. Out of about 160 and odd research papers published, more than half (i.e. 100 papers) cover these areas<sup>1-6</sup>. More comprehensive coverage is available in three collected volumes<sup>7-9</sup>, one of them a review article<sup>9</sup>.

### **(2) X-ray Analysis of Small Organic Biomolecules**

During the late fifties and early sixties, a large number of small molecules, mostly amino acids, were subjected to systematic X-ray analysis. This was a part of a major programme of research in the department on the structure of biological molecules, particularly proteins and other macromolecules. X-ray structure elucidation of the monomeric units paved the way for some of the most crucial conformational studies of biopolymers of the Madras School, pioneered by Prof. G N Ramachandran and coworkers. The Madras School is now internationally well known for these contributions. Incidentally, during this period, this group was also responsible for the largest number of molecule structures X-ray analysed in India. Even with rather primitive equipment, close to 200 structures, covering a variety of systems of biological interest, were established; about 40% of these involved work carried out by Srinivasan along with his students and colleagues.

### **(3) Biomacromolecules: Studies on Protein Folding**

Roughly from the middle of the seventies, studies on protein folding were initiated. The approach was to examine a



very large number of protein crystals, X-ray analysis data for which are available. While on the one hand conformational studies on biomolecular systems could be attempted from purely theoretical or semi-empirical approaches, a vast amount of X-ray crystallographic data that had accumulated over the years had not been made use of adequately in earlier studies. In trying to understand the protein folding features, this is of vital interest. The first paper on this type of work from the department<sup>10</sup> reported new powerful methods of unravelling the protein folding features and was based on virtual bond concept. This was followed by a series of papers<sup>11-13</sup>. These methods offer a powerful tool for studying the intricate details in helical regions in globular proteins. The 'best helical parameters' for the alpha helix were deduced<sup>12</sup>. Supercoiling noticeable in single constants of alpha helix in globular proteins and special helical distortions were also brought out<sup>14</sup>. Simplifying characterization of bend regions in proteins became possible through one of the new parameters proposed<sup>13</sup>. These are also reviewed in symposia proceedings<sup>15-17</sup>.

#### (4) Wideline NMR: Structural Applications

Wideline nuclear magnetic resonance spectroscopy in the solid state has an increasingly important role to play in structure elucidation of molecular systems in the solid state and is a vital adjunct to X-ray structural studies. This kind of work was also initiated roughly from the middle of 1975 and close to about 20 papers have been published in this area by Srinivasan and his colleagues. Some of the significant papers in this area are on line shape analysis using Fourier techniques<sup>18</sup> and

truncated moment analysis on line shape and its utility in deducing the presence of molecular motion<sup>19</sup> in the solid state. The combined use of wideline NMR and neutron diffraction data enabled important results to be deduced, for instance, the over-correction for the riding model of thermal vibration in structural crystallography<sup>20</sup>.

#### (5) Other Studies

Of late, Srinivasan has been interested in synchrotron radiation in crystallography, molecular biophysics and other related areas. An invited contribution<sup>21</sup> on synchrotron radiation was presented in 1980 at the National Seminar in Pune. An interesting possible experiment for the determination of sense of helical configuration in the solid state using anomalous scattering has been pointed out by him<sup>22</sup>. This and a few other possible diffraction experiments have been formulated, but these require synchrotron radiation facilities, which unfortunately are not currently available in India.

#### Selected Publications

1. Ramachandran G N & Srinivasan R, A new statistical test for distinguishing between centrosymmetric & non-centrosymmetric structures, *Acta Cryst*, **12** (1959) 410.
2. Srinivasan R, The significance of the phase synthesis, *Proc Indian Acad Sci*, **A53** (1961) 252.
3. Ramachandran G N & Srinivasan R, An apparent paradox in crystal structure analysis, *Nature, Lond*, **190** (1961) 159.
4. Ramachandran G N & Srinivasan R, X-ray intensity statistics of a pair of related crystals, *Nature, Lond*, **200** (1963) 1090.
5. Srinivasan R & Srikrishnan T, New statistical tests for distinguishing between centrosymmetric and non-centrosymmetric structures, *Acta Cryst*, **21** (1966) 648.
6. Srinivasan R & Chacko K K, Fourier treatment of the anomalous dispersion corrections in diffraction data, *Curr Sci*, **36** (1967) 279.

7. Srinivasan R, Application of X-ray anomalous scattering in structural studies, in *Advances in structural research by diffraction methods*, Vol 4, edited by W Hoppe and R Mason (Vieweg Verlag, Berlin) 1971, 105-97.
8. Ramachandran G N & Srinivasan R, *Fourier methods in crystallography* (John Wiley & Sons, New York) 1970, pp 257.
9. Srinivasan R & Parthasarathy S, *Some statistical applications in crystallography* (Pergamon Press, Oxford) 1976, pp 248.
10. Srinivasan R, Balasubramanian R & Rajan S S, Analysis of protein crystallographic structural data: Part I. Some new methods and general results, *J molec Biol*, **98** (1975) 739-47.
11. Srinivasan R, Balasubramanian R & Rajan S S, Analysis of protein crystallographic structural data: Part II. Analysis of  $\theta$  and delta parameters on six proteins, *J theor Biol*, **67** (1977) 299-312.
12. Rajan S S & Srinivasan R, Helical segment analysis of  $\alpha$ -helical regions in proteins, *Biopolymers*, **16** (1977) 1617-34.
13. Srinivasan R & Ravichandran V, Analysis of protein crystallographic structural data: Analysis of the orientation of peptide planes in proteins, *Int J biol Macromol*, **4** (1982) 211-14.
14. *Lectures delivered at the International Winter School on Current Trends in Biomolecular Structure, Madras, in January 1978*, edited by R Srinivasan & Vasantha Pattabhi (Macmillan India Ltd) 1981, pp 428.
15. *Biomolecular structure, conformation, function and evolution: Vol I--Diffraction and related studies*, edited by R Srinivasan, E Subramanian & N Yathindra (Pergamon Press, England) 1981, pp 685.
16. *Biomolecular structure, conformation, function and evolution: Vol II--Physico-chemical and theoretical studies*, edited by R Srinivasan, N Yathindra & E Subramanian (Pergamon Press, England) 1981, pp 653.
17. *Advances in crystallography*, edited by R Srinivasan (Oxford & IBM Publishing Company) 1978, pp 222.
18. Ramani K, Ganapathy S & Srinivasan R, A Fourier transform approach to lineshape analysis and application to NMR, *J mag Res*, **24** (1976) 231-37.
19. Srinivasan R & Jagannathan N R, The use of truncated moments for the lineshape analysis in wideline NMR, *Indian J pure appl Phys*, **20** (1982) 879-83.
20. Srinivasan R & Jagannathan N R, On the riding model correction for bond lengths, *Acta Cryst*, **B38** (1982) 2093-95.
21. Srinivasan R, *Advances in biocrystallography and molecular biophysics using synchrotron radiation*, Paper presented at Seminar on Applications on Synchrotron Radiation, Poona, 1984.
22. Srinivasan R & Santhanam P, *The effect of X-ray anomalous scattering on helical diffraction*, Collected abstracts of the International Symposium on Biomolecular structure, conformation, function & evolution, Madras, S219, 1978.



## Joseph Thomas

During doctoral work at the Institute of Science, Bombay, on the taxonomy and ecophysiology of the algae of hot springs, I became interested in the biology of  $N_2$ -fixing cyanobacteria, then called blue-green algae. But it was some years before I could do research on  $N_2$  fixation. After a post-doctoral year at the Institute of Microbiology, Prague, I joined Bhabha Atomic Research Centre (BARC), Bombay. Modifying a microspectrophotometer, a device originally designed for making absorption measurements on animal cell organelles, we used analogue computer techniques to record the first derivative of absorption of the chloroplasts of single algal cells<sup>1</sup>. Several long wavelength forms of chlorophyll *in vivo* were resolved, including  $C_{690}$  and  $C_{700}$ , two key pigment molecules in the energy transfer chain of photosynthesis.

### Heterocysts of Cyanobacteria as Sites of Nitrogen Fixation

The experience with microspectrophotometry turned out to be even more useful when I began  $N_2$  fixation research. At that time, there was great interest in the role of heterocysts, specialized cells which differentiate during nitrogen deficiency in many filamentous cyanobacteria<sup>2</sup>. Measurements of individual heterocysts in several hundred filaments revealed that newly differentiated heterocysts lack or

have very little c-phycocyanin, allophycocyanin, c-phycoerythrin and  $C_{670}$ , the shortwave form of chlorophyll  $a^3$ . This was the first direct evidence indicating that these energy harvesting pigments of photosystem II of photosynthesis involved in  $O_2$  evolution are absent in heterocysts. More extensive microspectrophotometric study<sup>4</sup> followed by fluorescence emission spectral examination of isolated heterocysts at 77°K confirmed the absence of PS II. These findings demonstrated that heterocysts are ideal sites, providing the anaerobic milieu essential for  $O_2$ -sensitive nitrogenase, to catalyse the reduction of  $N_2$  to ammonia. This was substantiated<sup>5</sup> by cytochemically locating the enzyme activity in heterocysts and by correlating the appearance and abundance of heterocysts with nitrogenase activity, determined by the newly discovered acetylene reduction assay.

### The Initial Pathway of Radioactive Nitrogen ( $^{13}N$ ) Metabolism

Although the foregoing researches from my laboratory and the findings of a few other workers in Europe and America well nigh established the role of heterocysts, no one had yet demonstrated the actual reduction of  $N_2$  to ammonia in heterocysts. While on sabbatical leave in Peter Wolk's laboratory at Michigan State University, I developed techniques to

---

Head, Biological Nitrogen Fixation Section, Molecular Biology and Agriculture Division, Bhabha Atomic Research Centre, Trombay, Bombay-400085; Residence : 20A, Dhawalgiri, Anushaktinagar, Bombay-400095.

rapidly process microbes labelled with radioactive nitrogen ( $^{13}\text{N}$ ;  $t_{1/2}$  10 min) gas and to isolate physiologically active heterocysts under anaerobic conditions stringent enough to protect nitrogenase. We then unequivocally demonstrated that  $\text{N}_2$  is reduced to ammonia in heterocysts and that nitrogenase is coupled in the same cells to glutamine synthetase (GS), leading to the formation of glutamine<sup>6</sup>.

The  $^{13}\text{N}$ -labelling methodology enabled us to trace, in spite of the 10 min half-life of the isotope, the initial pathway of nitrogen metabolism in cyanobacteria<sup>7,8</sup>. The work established the predominant role of the GS-GOGAT (glutamine amide oxoglutarate aminotransferase) pathway in cyanobacteria and led to the confirmation of this pathway in legume nodules and plant cell cultures.

### Identification and Cloning of Genes Regulating Nitrogen Metabolism

The work on the initial pathway of nitrogen metabolism developed, on my return to India, into a wider interest in GS, the key enzyme of nitrogen metabolism. A facile affinity chromatographic method for the purification and quantitative recovery of the enzyme from *Anabaena* L-31 enabled its biochemical characterization and helped reveal its multiple regulation *in vivo*. In the presence of ammonium, repression of GS leads to a decrease in the overall enzyme activity to only half the level in  $\text{N}_2$ -grown cultures. There is also ammonium mediated modification of the enzyme which retains the earlier affinity for substrates, but shows depression in  $V$  leading to drastically reduced specific activity<sup>9</sup>. Additional modulation is effected by cumulative inhibition by amino acids, which can be reversed by glyoxylate, an intermediate in photorespiration<sup>10</sup>.

Two properties of cyanobacterial GS observed by us, which are strikingly different from those of bacterial GS, proved to be very valuable and eventually led to the invalidation of the prevalent central dogma on nitrogen regulation. It was propounded that unadenylylated GS is the positive activator of transcription of several genes involved in the synthesis of enzymes and other proteins having a crucial role in nitrogen metabolism. We found that cyanobacterial GS is not subject to adenylation cascade. Moreover, the half-life of GS in the presence of ammonium is nearly 24 h, whereas that of nitrogenase is less than 4 h, indicating a lack of correlation between the expression of these enzymes<sup>9,10</sup>.

The regulatory role of GS was then examined by molecular genetic analyses in enteric bacteria. Recombinant DNA plasmids containing inserts from the *glnA* (GS structural gene) region of *Escherichia coli* were used to study the expression of *gln*, *hut* (histidine utilization) and *nif* operons in a regulation defective mutant ( $\text{Gln}^- \text{Hut}^- \text{Nif}^-$ ) of *Klebsiella pneumoniae*, K.P. 5060. Genes adjacent to the C-terminal end of *glnA* on the *E. coli* chromosome were able to derepress *hut* and *nif* operons in *K. pneumoniae* in the absence of *glnA* product (GS). Complete derepression of *nif* operons required inclusion of the segment adjacent to the N-terminal end of the *glnA* region of *E. coli* chromosome along with the C-terminal end segment. In the absence of GS, *nif* was not only fully derepressed but also constitutive. To conclude, *glnA* product (GS) is not involved in the regulation of transcription of *nif* and other related operons, but DNA segments located adjacent to *glnA* now referred to as *ntr*



(nitrogen regulation) B and *ntnC* are the regulatory genes.

### Requirement of Sodium for Cyanobacterial Nitrogen Fixation

A major facet of the regulation of cyanobacterial  $N_2$  fixation emerged from our finding that in the absence of sodium,  $N_2$  fixation does not occur in cyanobacteria<sup>12,13</sup>.  $Na^+$  is required for the efficient uptake of phosphate from the medium to synthesize sufficient nucleoside phosphates (ATP). Adequate levels of nucleoside phosphates seem to be essential for processing nitrogenase to reduce  $N_2$  to ammonia.

### Salt Tolerance in Cyanobacteria as Effected by Modulations of Sodium Transport

Nitrogen fixing cyanobacteria are capable of profuse growth in saline/alkali soils and their use has been advocated for reclamation of such soils. In recent studies<sup>13</sup>, we have been able to unravel some of the basic features of this salt tolerance.  $Na^+$  influx in *Anabaena torulosa* and *Anabaena* L-31, a brackish water and fresh water cyanobacterium respectively, is unaffected by low  $K^+$  concentration, is carrier mediated, and is modulated mainly by membrane potential. Both cyanobacteria also exhibit rapid active efflux of  $Na^+$ . Higher affinity of carrier for  $Na^+$  and capability to curtail  $Na^+$  influx and promote  $Na^+$  efflux enable *A. torulosa* to be more salt tolerant.

### Early Interactions in Legume-Rhizobium Symbiosis

To improve the efficiency of biological nitrogen fixation and to extend the property to other crops, it is important to understand the processes by which legume and rhizobium recognize each

other and interact during the early steps of the symbiosis.

Using cowpea (*Vigna unguiculata*) we have identified some components of the likely sequence of early interactions<sup>14,15</sup>. Cowpea roots secrete, especially during nitrogen deficiency, substances which cause the existing polysaccharide envelope (capsular polysaccharides, CPS) of its specific *Rhizobium* partner to change its physical and chemical structure. Instead of two native CPS components, the host induced CPS (HI-CPS) separate into three components on cellulose membrane electrophoresis or on DEAE-Sephadex chromatography. On interaction with root exudate, the proportions of constituent sugars of CPS are altered and arabinose and xylose appear as new constituents. Addition of HI-CPS to cowpea roots induces *de-novo* synthesis of root wall proteins and root hair curling followed by enhanced nodulation efficiency.

### Selected Publications

1. Thomas J, Phondke G P, Tatake V G & Gopal-Ayengar A R, Microspectrophotometric studies on the pigments *in vivo* of single algal cells. I. Pigments of *Chlorella pyrenoidosa*, *Photochem Photobiol*, **11** (1970) 85-92.
2. Thomas J & David K A V, Studies on the physiology of heterocyst production in the nitrogen-fixing blue-green alga *Anabaena* sp. L-31 in continuous culture, *J gen Microbiol*, **65** (1971) 127-31.
3. Thomas J, Absence of the pigments of photosystems II of photosynthesis in heterocysts of a blue-green alga, *Nature, Lond*, **228** (1970) 181-83.
4. Thomas J, Relationship between age of culture and occurrence of the pigments of photosystem II of photosynthesis in heterocysts of a blue-green alga, *J Bact*, **110** (1972) 92-95.
5. Thomas J & David K A V, Site of nitrogenase activity in the blue-green alga *Anabaena* sp. L-31, *Nature, Lond*, **238** (1972) 219-21.
6. Thomas J, Meeks J, Wolk C P, Shaffer P W, Austin S M & Chien W S, Formation of

- glutamine from ( $^{13}\text{N}$ ) ammonia, ( $^{13}\text{N}$ ) dinitrogen, and ( $^{14}\text{C}$ ) glutamate by heterocysts isolated from *Anabaena cylindrica*, *J Bact*, **129** (1977) 1545-55.
7. Thomas J, Wolk C P, Shaffer P W, Austin S M & Galonsky A, The initial organic products of fixation of  $^{13}\text{N}$ -labelled nitrogen gas by the blue-green alga *Anabaena cylindrica*, *Biochem biophys Res Commun*, **67** (1975) 501-7.
  8. Wolk C P, Thomas J, Shaffer P W, Austin S M & Galonsky A, The pathway of nitrogen metabolism after fixation of  $^{13}\text{N}$ -labelled nitrogen gas by the cyanobacterium, *Anabaena cylindrica*, *J biol Chem*, **251** (1976) 5027-34.
  9. Tuli R & Thomas J, *In vivo* regulation of glutamine synthetase by ammonium in the cyanobacterium *Anabaena* L-31, *Archs Biochem Biophys*, **206** (1981) 181-89.
  10. Tuli R & Thomas J, Regulation of glutamine synthetase in the blue-green alga *Anabaena* L-31, *Biochim biophys Acta*, **613** (1980) 526-33.
  11. Tuli R, Iyer R K & Thomas J, Regulation of expression of *Nif* and *hut* operons in *Klebsiella pneumoniae* by *glnA* linked genes of *Escherichia coli*, *Molec gen Genet*, **187** (1982) 342-46.
  12. Apte S K & Thomas J, Effect of sodium on nitrogen fixation in *Anabaena torulosa* and *Plectonema boryanum*, *J gen Microbiol*, **130** (1984) 1161-68.
  13. Thomas J & Apte S K, Sodium requirement and metabolism in nitrogen fixing cyanobacteria, *J Biosci*, **6** (1984) 771-94.
  14. Bhagwat A A & Thomas J, Legume-Rhizobium interactions: Cowpea root exudate elicits faster nodulation response by *Rhizobium* species, *Appl Env Microbiol*, **43** (1982) 800-5.
  15. Bhagwat A A & Thomas J, Legume-Rhizobium interactions: Host induced alterations in capsular polysaccharides and infectivity of cowpea rhizobia, *Arch Microbiol*, **140** (1984) 260-64.



## C S Vaidyanathan

The investigations carried out by our group during the past two decades were concerned mainly with the metabolism of phenolic and other aromatic compounds in plants and fungi and have led to the discovery of several new reaction pathways involving these substances.

Anthranilic acid, an important intermediate of tryptophan metabolism, was found to be oxidized to catechol by a multi-enzyme system obtained from plant sources. In this connection, a highly sensitive colorimetric method for the estimation of catechol was developed<sup>1</sup>. Further investigations led to the resolution of this multi-enzyme system into three well-defined component enzymes. The overall reaction catalyzed by these component fractions is the oxidation of anthranilic acid to catechol via intermediates like 3-hydroxyanthranilic acid and o-aminophenol.

During the course of these investigations, two new enzyme systems, one catalyzing the conversion of o-aminophenol to isophenoxazine and the other involved in the conversion of isophenoxazine to catechol<sup>2</sup> were discovered. It was further demonstrated that 3-hydroxyanthranilic acid is oxidized to cinnabaric acid by an enzyme system present in the nuclear fraction of rat liver and also in the leaves of *Tecoma stans*.

Evidence that indole is one of the precursors of anthranilic acid in *Tecoma* leaves has been obtained. The presence of an indole oxidase in the leaves of *Tecoma stans* was demonstrated<sup>3</sup>. The end-product of the reaction in the presence of a partially purified enzyme preparation was identified as anthranil. Formylaminobenzaldehyde and o-aminobenzaldehyde were detected as intermediates in the overall conversion. The enzyme showed an absolute requirement of FAD and Cu<sup>2+</sup> for maximum activity. Inhibition studies with GSH and PCMB showed that a sulphhydryl-cupric ion complex at the active centre is essential for enzyme activity.

### Anthranilate Hydroxylase (Double Hydroxylating) from *A. niger*

A new anthranilic acid hydroxylase which catalyzes the conversion of anthranilic acid to 2,3-dihydroxybenzoic acid has been purified to homogeneity from the mycelial extracts of *A. niger* grown in the presence of anthranilic acid<sup>4</sup>.

The properties of this fungal enzyme indicated that it is entirely different from the dihydroxylating enzymes of bacterial systems. To substantiate this fact, the reaction catalyzed by the enzyme was conducted in <sup>18</sup>O<sub>2</sub>-enriched atmosphere and the enzyme was shown to be a mono-oxygenase. This enzyme, unlike other

---

Professor, Department of Biochemistry, Indian Institute of Science, Bangalore-560012;  
Residence : 13, Duplex Quarters, Indian Institute of Science Campus, Bangalore-560012.

mono-oxygenases, was solely dependent on NADPH and hence represents a new class of mono-oxygenases.

Using initial velocity and product inhibition, the kinetic mechanism of this novel enzyme has been elucidated and the reaction was shown to follow Bi Uni Uni Bi ping pong Ter Ter mechanism.

From the ORD and CD spectra, the helical content of the enzyme was calculated to be about 20%.

The participation of iron in this double hydroxylation reaction has been confirmed by ESR studies on the bound  $\text{Fe}^{2+}$  in the enzyme. The native enzyme showed no signal from  $g$  value 1.90-6.0. On the addition of anthranilic acid, an asymmetric signal was observed at  $g = 4.3$ , characteristic of high spin  $\text{Fe}^{3+}$ . On the addition of NADPH, the asymmetric signal at  $g = 4.3$  changed to a symmetric form, due to a change in the ligand field around the bound iron. These observations suggest that the enzyme has bound  $\text{Fe}^{2+}$ , which gets oxidized to  $\text{Fe}^{3+}$  during catalysis.

### New Metabolic Reactions of Catechol in Plants

A wide range of diverse natural products found in higher plants and micro-organisms (lignins, tannins, antibiotics and alkaloids) are recognized to be derived from hydroxy aromatic compounds by oxidative coupling involving C-C and C-O linkages. It was interesting to see whether such oxidation products were formed from catechol in plant systems. It was observed by us that leaf extracts of *Tecoma* can indeed carry out the oxidation of catechol to yield diphenylene dioxide-2,3-quinone (DDQ) and 3,4,3',4'-tetrahydroxydiphenyl (THDP). Two distinct enzymes, one

involved in the conversion of catechol to DDQ and the other catalyzing the formation of THDP have been purified to homogeneity and a detailed study has been made of the properties and kinetics of these new enzymes<sup>5,6</sup>. The formation of C-C and C-O linked oxidation products in these reactions could be explained on the basis of the formation of aryloxy radicals.

### Aromatic Ring Fission in Plants

Suggestive evidence for the occurrence of aromatic ring fission in higher plants has been mounting in recent years. However, no report on studies with isolated enzyme systems has appeared so far. We have been successful in the detection and purification of an enzyme system from *Tecoma* leaves which catalyses the oxidation of 2,3-dihydroxybenzoic acid, leading to the isolation of an aliphatic product which reacted as a dilactone<sup>7</sup>. This has been characterized as 2,5-dioxo-3,7-dioxo bicyclo (3:3:0) octane-8-carboxylic acid by chemical and physical methods. An intra-diol oxygenative cleavage of 2,3-dihydroxybenzoic acid was proposed to account for its formation. This is the first report on aromatic ring fission by an isolated enzyme system from a plant source.

### Selected Publications

1. Madhusudanan Nair P & Vaidyanathan C S, A colorimetric method for the determination of pyrocatechol and related substances, *Analyt Biochem*, **7** (1964) 315.
2. Madhusudanan Nair P & Vaidyanathan C S, The conversion of isophenoxazine to catechol in *Tecoma stans*, *Archs Biochem Biophys*, **115** (1966) 515.
3. Madhusudanan Nair & Vaidyanathan C S, An indole oxidase from *T. stans*, *Biochim biophys Acta*, **81** (1964) 496.
4. Sreeleela N S, Subba Rao P V, Prema Kumar R & Vaidyanathan C S, A new anthranilic acid



- hydroxylase from *A. niger*; purification and properties, *J biol Chem*, **244** (1969) 2293.
5. Kandaswami C & Vaidyanathan C S, Oxidation of catechol in plants. III. Purification and properties of the diphenylene dioxide 2,3-quinone forming enzyme system from *Tecoma* leaves, *Archs Biochem Biophys*, **155** (1973) 247.
6. Kandaswami C & Vaidyanathan C S, Oxidation of catechol in plants. IV. Purification and properties of 3,4,3',4'-tetrahydroxy diphenyl-forming enzyme system from *Tecoma* leaves, *J biol Chem*, **248** (1973) 4035.
7. Sharma H K & Vaidyanathan C S, A new mode of ring cleavage of 2,3-dihydroxy benzoic acid in *Tecoma stans* L., *Eur J Biochem*, **56** (1975) 163.

**Sectional Committee—X : Agriculture,  
Animal Husbandry, Fisheries and Forestry**





## I P Abrol

Soil salinization and alkalization are worldwide phenomena resulting in the degradation of productive lands, making them unfit for agricultural purposes. These processes are most serious in the sub-humid, semi-arid and arid regions. Land resources being limited, it is urgent that the productivity of lands that have gone out of cultivation is restored and steps are taken to prevent further deterioration of this basic resource.

There are an estimated 7 million hectares of salt affected soils in India producing little or no crops. Alkali soils, constituting a large fraction of these, are widespread in the Indo-Gangetic plains in the states of Haryana, Punjab, Uttar Pradesh, Bihar, Madhya Pradesh and to a somewhat lesser degree in other parts of the country. Such soils are also widespread in several parts of the world.

Intensive basic researches by Abrol over the past two decades have provided a better understanding of the nature and characteristics of alkali soils; factors limiting production and management have been identified and in-depth studies carried out to get pointed solutions to the problem, resulting in savings in the reclamation costs and the development of sound management practices for the utilization of these soils for agriculture and

agro-forestry. The following conclusions have emerged from these researches.

### (1) Characteristics and Diagnosis

(i) The characteristics of alkali soils have been elucidated and it has been concluded that these soils tend to occur in geographic zones distinct from those where the saline soils tend to dominate. The soils are characterized by the dominance of sodium bicarbonate and carbonates among the soluble salts, resulting in high exchangeable sodium percentage in the profile. The regions where alkali soils dominate are generally associated with ground waters of low total salinity and mild alkalinity.

(ii) A fresh look has been provided on the diagnostic criteria and nomenclature of salt affected soils. It has been shown that there is a definite relation between soil pH and sodicity and that the relationship is theoretically sound. It has been further shown that a saturation paste pH of 8.2 is more realistic for differentiating alkali from non-alkali soils than the hitherto used value of 8.5 suggested by US workers. A further classification of soils into varying ESP and pH classes in relation to their sodicity hazard has been suggested. These results have provided methodology for a more realistic assessment of the field problems and this, in turn, has enabled the



adoption of appropriate corrective measures.

(iii) Critical studies have shown that alkali soils have a pH dependent component of charge that contributes to their enhanced cation exchange capacity. For this reason, it has been shown that the existing laboratory methods of determining cation exchange capacity and exchangeable sodium in alkali soils are in error. A new method for evaluating these two important and basic soil parameters has been suggested. In-depth studies have been carried out to bring out the relevance of pH dependent charge on the management requirement of these soils.

## **(2) Management**

Studies of Abrol have enabled optimum practices for the management of these soils to be defined.

### **(i) Amendments**

(a) A new method has been suggested for determining the gypsum requirement of alkali soils. It has been shown that the existing procedure overestimates the gypsum requirement of alkali soils containing free sodium carbonate and that the modified procedure provides a more realistic value.

(b) The comparative performance of several amendments for alkali soil reclamation has been evaluated.

(c) The optimum size and method of application of amendments have been defined.

(d) The chemistry of fluorine in alkali soils and its effect on plant growth have been elucidated. These studies have formed the basis for defining the upper permissible limit of fluorine in byproduct

gypsum available in large quantities for use as an amendment. These studies have formed the basis for framing the ISI specifications for byproduct gypsum.

### **(ii) Crop Tolerance**

The relative tolerance of several field crops to varying levels of soil sodicity has been defined. The results of these studies have formed the basis for making practical recommendations relating to the selection of crops and optimum cropping sequence for alkali soils. It has been further shown that rice crop grown in alkali soils exerts a marked reclaiming effect. The possible mechanisms responsible for this have been elucidated.

### **(iii) Physical Properties of Soil and Water Management**

Quantitative information has been obtained on the relationship between exchangeable sodium and the important physical properties of soil. Since the adverse effect of exchangeable sodium on plant growth is mediated chiefly through its effect on the physical properties of soil, including their water relationship, the influence of exchangeable sodium on soil water behaviour has been studied under field and laboratory conditions. The effect of soil sodicity on water uptake by crops was studied under field conditions through continuous monitoring of water content and flux changes with time and depth. These studies have brought out a clear understanding of the water management needs of crops grown in alkali soils.

### **(iv) Agro-forestry in Alkali Soils**

A large fraction of alkali lands form a part of the village common lands which are ideally suited for agro-forestry. The 'Auger hole technique' developed by Abrol has

opened new vistas for planting trees in these soils. Similarly, highly tolerant grasses have been identified which can be grown without application of amendments. Growing of grasses and trees together is an excellent alternative use for alkali lands. Such a land use has been shown not only to provide the much needed fodder and fuel for the villages, but also to enable environmental improvement and conservation of soil and water resources.

### (3) Impact of Research

The researches of Abrol have resulted in economically viable and technically sound technologies for the reclamation of alkali soils. The technologies have been demonstrated widely and accepted by the farmers. The state governments of Haryana, Punjab and Uttar Pradesh have set up Land Reclamation Corporations to assist the farmers in reclaiming their lands which have hitherto been lying barren for decades. Through the adoption of these technologies and the infrastructure provided by the Corporations, in the past 8-10 years, the farmers have reclaimed several hundred thousand hectares of alkali lands which are now contributing more than 2 million tonnes of additional foodgrains annually. In the next 7-8 years, the central government has planned to assist the state governments in reclamation of at least 1 million ha land. This will give additional food production of nearly 6 million tonnes annually. Apart from the gains in food production, there are also benefits like environmental improvement, conservation of rain water, reduction in flood hazards, etc. Reclamation of these lands will also generate permanent employment potential to the extent of about 150 man-days per

year for each hectare brought under cultivation. The improved planting techniques evolved for trees and highly tolerant grasses identified as suitable for growing under alkali conditions will go a long way in restoring the degraded lands for forage and fuel production from these wasted lands.

### Selected Publications

1. Abrol I P, Dahiya I S & Bhumbra D R, On the method of determining gypsum requirement of soils, *Soil Sci*, **120** (1975) 30-36.
2. Abrol I P & Bhumbra D R, Crop response to differential gypsum applications in a highly sodic soil and the tolerance of several crops to exchangeable sodium under field conditions, *Soil Sci*, **127** (1979) 71-85.
3. Acharya C L, Sandhu S S & Abrol I P, Effect of exchangeable sodium on the rate and pattern of water uptake by *Raya (Brassica juncea)* under field conditions, *Agron J*, **71** (1979) 936-41.
4. Acharya C L, Sandhu S S & Abrol I P, Effect of exchangeable sodium on the field drying pattern of soil at two evaporative demands, *Soil Sci*, **127** (1979) 56-62.
5. Khosla B K, Gupta R K & Abrol I P, Studies on salt leaching and the effect of gypsum application in a saline-sodic soil, *Agric Wat Mgmt*, **2** (1979) 193-202.
6. Chhabra R, Singh A & Abrol I P, Fluorine in sodic soils, *Soil Sci Soc Am J*, **44** (1980) 33-36.
7. Gupta R K, Chhabra R & Abrol I P, Relationship between pH and exchangeable sodium in a sodic soil, *Soil Sci*, **131** (1981) 215-19.
8. Ashok Kumar & Abrol I P, Relative performance of some forage grasses in relation to soil pH and sodicity, *Forage Res*, **8** (1982) 127-31.
9. Chawla K L & Abrol I P, Effect of gypsum fineness on the reclamation of sodic soils, *Agric Wat Mgmt*, **5** (1982) 41-50.
10. Gupta R K, Chhabra R & Abrol I P, Fluorine adsorption behaviour in alkali soils: Relative roles of pH and sodicity, *Soil Sci*, **133** (1982) 364-68.
11. Ashok Kumar & Abrol I P, Effect of gypsum on five tropical grasses grown in normal and extremely sodic soil, *Exp Agric*, **19** (1983) 169-77.
12. Gupta R K, Bhumbra D K & Abrol I P, Effect of sodicity, pH, organic matter and CaCO<sub>3</sub> on the dispersion behaviour of soils, *Soil Sci*, **137** (1984) 245-51.



13. Gupta R K, Bhumbra D K & Abrol I P, Release of exchangeable sodium from an alkali soils upon amendment application—Role of variable charge and exchangeable cation hydrolysis, *Soil Sci*, **139** (1985) 312-18.
14. Gupta R K, Singh C P & Abrol I P, Determination of cation exchange capacity and exchangeable sodium in alkali soils, *Soil Sci*, **139** (1985) 326-32.
15. Abrol I P & Sandhu S S, Growth responses of *Eucalyptus tereticornis* and *Acacia nilotica* to selected methods of site preparation in a highly sodic soil, *Int Tree Crop J*, **3** (1985) 171-83.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

## Akhtar Husain

After obtaining his Master's degree in agriculture from the Government Agriculture College, Kanpur, in 1954, Husain got a research fellowship from the North Carolina State University (USA), from where he secured his Doctor's degree in plant pathology in 1957; he was selected as the most outstanding PhD student of that university for the year 1957 and received the Phi Kappa Phi award. He worked as Assistant Plant Pathologist in the Connecticut Agricultural Experimental Station, New Heaven (USA) for about a year, and made a notable contribution to the physiology of parasitism of Dutch elm disease and tomato wilt. While at New Heaven, Akhtar Husain contributed, on invitation, a chapter in one of the volumes of *Advanced Treatise on Plant Pathology* (Academic Press, New York).

After his return to India in 1958, Husain joined ICAR as Plant Pathologist at the Regional Research Centre (Oilseeds and Millets) at Kanpur and became Head of the Centre in 1961. He made notable contributions to the control of diseases of oilseeds and millets.

Husain joined the Regional Research Laboratory (RRL), Jammu and Kashmir in 1963. At this laboratory, he first worked as Head of the Division of Plant Protection and later organized the Discipline of Agricultural Sciences. At RRL, Jammu,

Husain was also Scientist-in-Charge of the Drug Farms and Factories (CIMAP) for more than three years. It was during this period that the Farms and Factories (CIMAP) achieved a breakthrough and the unit, which was undergoing heavy losses for more than 13 years, became a profit-making undertaking.

As a result of R & D efforts carried out under Husain's guidance, belladonna (*Atropa belladonna*) was introduced on a large scale on Kashmir Farms and the country became self-sufficient in this drug within three years. He was responsible for the introduction of hops as a commercial crop on CIMAP Farms and also started the first experiments on the cultivation of *Dioscorea deltoidea* on the farms.

Husain joined CIMAP in September 1975, and became Director of this Institute in May 1977. One of the main contributions of Husain and his team is the development of indigenous technology for artificial cultivation of ergot of rye (*Claviceps purpurea*) in India. As a result, ergot is being grown commercially in the country, making it self-sufficient in this valuable drug, earlier imported from Europe. Husain and his coworkers were able to develop four new strains containing predominantly ergotamine, ergotoxine, ergokryptine and ergometrine. Studies undertaken on the cultivation of ergot



indicated that Palni Hills of Tamil Nadu are an ideal location for profitable cultivation of ergot. Further, it was possible to get two crops of ergot in a year in this area. This is the first record of cultivation of two crops of ergot in a year anywhere in the world.

Recently, Husain and his team have made a major breakthrough in developing a new strain of *Claviceps paspali* Stevens & Hall producing ergot alkaloids in submerged culture. A new chemical race giving significant yield of ergometrine in culture broth was isolated. Total alkaloids of one of the strains contained 25.7% ergometrine, 20.5% lysergic acid amide, 18.4% ergometrinine and 8.5% lysergic acid. Lysergic acid and lysergic acid amide are important precursors for the synthesis of therapeutically important ergot alkaloids. A number of pharmaceutical firms have developed strains which produce lysergic acid and lysergic acid amide in culture, but development of a strain producing ergometrine had not been reported earlier.

Under his guidance, the institute has been able to develop modern distillation technology for rose oil and to evolve improved strains of Japanese mint, opium poppy and lemongrass.

### Selected Publications

1. Husain A & Kelman A, Presence of pectic and cellulolytic enzymes in plants infected by *Pseudomonas solanacearum*, *Phytopathology*, **47** (1957) 111-12.
2. Husain A & Kelman A, Relation of slime production to mechanism of wilting and pathogenicity of *Pseudomonas solanacearum*, *Phytopathology*, **48** (1958) 155-65.
3. Husain A & Kelman A, Role of pectic and cellulolytic enzymes in pathogenesis of *Pseudomonas solanacearum*, *Phytopathology*, **48** (1958) 377-85.
4. Husain A & Dimond A E, The function of extracellular enzymes of the Dutch elm disease pathogen, *Proc natn Acad Sci, USA*, **44** (1958) 594-601.
5. Husain A & Kelman A, Tissue is disintegrated, in *Plant pathology : An advanced treatise*, Vol 1, edited by James Horsfall and A E Dimond (Academic Press, New York) Chap 5.
6. Husain A & Dimond A E, Role of cellulytic enzymes in pathogenesis—*Fusarium oxysporum f. lycopersici*, *Phytopathology*, **50** (1960) 329-31.
7. Husain A & Singh K P, Commercial production of pharmaceutical grade of ergot in India, *Labdev J Sci Technol*, **6** (1968).
8. Husain A & Janardhanan K K, Role of toxins in plant diseases, *Glimpses Pl Sci*, **3** (1976) 148-86.
9. Husain A, Singh K P & Thakur R N, Commercial production of ergot in India, *Indian Drugs*, **13**(5,6) (1976) 529.
10. Tajuddin Singh A, Ram Paltoo & Husain A, Introduction of hops as a commercial crop in Kashmir Valley, *New Botanist*, **4**(1-4) (1977) 37-39.
11. Janardhanan K K, Singh K P & Husain A, Introduction of ergotoxine strain of ergot in India, *Indian J pharm Sci*, **40**(6) (1978) 210-11.
12. Janardhanan K K & Husain A, Production of morel mushroom mycelium : A potential source of protein and flavour, in *Indian mushroom science*, I, edited by C K Atal, B K Bhat and T N Kaul (Indo-American Literature House, New Delhi) 1978, 495-508.
13. Husain A, Singh P & Singh A, Introduction of a high alkaloid strain of *Hyoscyamus muticus* in India, *Indian J pharm Sci*, **41** (1979) 46-48.
14. Husain A, Current status of essential oil industry in India, *PAFAI J*, **2**(2) (1979) 30-39.
15. Virmani O P, Singh P & Husain A, Current status of medicinal plants industry in India, *Indian Drugs*, **17**(10) (1980) 318-40.
16. Janardhanan K K, Gupta M L & Husain A, A new commercial strain of ergot adopted from a wild grass, *Plant Med*, **44** (1982) 166-67.
17. Husain A & Sharma J R, *The opium poppy—Medicinal and Aromatic Plants Series-I*, edited by a Husain and J R Sharma (CIMAP, Lucknow) 1983, pp. 167.
18. Janardhanan K K & Husain A, Studies on isolation, purification and identification of tenuazonic acid, a phytotoxin produced by *Alternaria alternata* (Fr) Keissler causing leaf blight of *Datura innoxia* Mill, *Mycopathologia*, **83** (1983) 135-40.

19. *Recent advances in plant pathology*, edited by A Husain, Kishan Singh, B P Singh & V P Agnihotri [Print House (India), Lucknow] 1983, 136-58.
20. Husain A, Sharma J R, Puri H S & Tyagi B R, Genetic resources of important medicinal and aromatic plants in South Asia—A *Status Report*, International Board for Plant Genetic Resources, Rome (Italy), CIMAP, Lucknow, 1984, pp. 326.
21. Janardhanan K K, Sattar A & Husain A, Production of fumigaclavine A by *Aspergillus tamarii* Kita, *Can J Microbiol*, **30** (1984) 247-50.
22. Janardhanan K K & Husain A, A new strain of *Claviceps paspali* Stevens & Hall producing ergometrine in submerged culture, *Proc Indian natn Sci Acad*, **85**(4) (1984) 438-40.
23. Janardhanan K K & Husain A, Phytotoxic activity of tenuazonic acid isolated from *Alternaria alternata* (Fr.) Keissler causing leaf blight of *Datura innoxia* Mill and its effect on host metabolism, *Phytopath Z*, **111** (1984) 305-11.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.



# I S Bhatia

## Plant Biochemistry

**Fructosans and their biosynthesis:** The presence of a series of homologous glucofructosans starting with sucrose molecule and ending up with polymeric fructosan was established in *Agave versa* Cruz. A rational scheme for the biosynthesis of these oligosaccharides was proposed involving the enzyme transfructosidase. Later, *in vitro* biosynthesis of glucofructosans in *Agave* up to F<sub>10</sub>G was achieved and a mechanism proposed. *In vivo* biosynthesis using discs of *Agave* stem has also been achieved. The presence of a fructosyl transferase was also established in chicory roots and the *in vivo* biosynthesis of glucofructosans at the expense of sucrose was suggested from data on water soluble carbohydrates during the growth of this plant.

A three-site active centre, one site each for the donor and acceptor sucrose molecules and the third for a nucleotide has been proposed in *Fusarium oxysporum*. The transfer of a fructose molecule from the donor to the acceptor sucrose is mediated through the nucleotide bridge. A close relationship between transfructosidase and invertase has been suggested, the primary difference between the two being in the degree of phosphorylation of the two enzymes.

**Starch and cellulose biosynthesis in cereals:** Experiments with detached heads of *Sorghum vulgare* incubated in labelled sugars indicated that compared to sucrose and fructose, <sup>14</sup>C was more efficiently incorporated from glucose into grain starch, which was maximally synthesized at midmilky stage of grain development. The decline in the rate of starch accumulation did not synchronize with that of protein synthesis.

A study of changes in the carbohydrates of bajra (*Pennisetum typhoides*), jowar (*Sorghum vulgare*) and kangni (*Setaria italica*) during growth showed that the water soluble carbohydrates initially increase in the stem, attaining a maximum value at flowering and initiation of seed formation, and, thereafter, start declining and reach a low value at maturation. By the early milk stage, most of the cellulose has already been formed and with the grain formation, the synthesis of cellulose slows down and the available carbohydrates are diverted primarily for the synthesis of grain carbohydrates.

## Lipids

(a) **Lipid metabolism in maturing and germinating oilseeds:** In taramira, the major change in the fatty acid composition was observed during 10-20 days after fertilization when there was rapid rise in

the oil content. A continuous rise in linoleic and linolenic acids with a concomitant fall in the relative amount of palmitic acid with seed maturity was observed. In cottonseed, during the stage of rapid fat synthesis, immediate desaturation of palmitic and stearic to oleic and linoleic acids has been postulated.

During germination of taramira, the triglycerides were degraded and it was shown that erucic acid was preferentially incorporated into the newly synthesized triglycerides. A limited synthesis of fatty acids needed for the formation of polar lipids of membranes has been demonstrated in germinating sunflower seed.

A constant decrease in the activity of acetyl CoA carboxylase was observed in germinating embryos of sunflower seeds. Exposure of etioplasts to light reduced the activity of the enzyme in the embryos. A reverse trend was observed in ripening embryos. The relationship of photophosphorylation and carboxylation with fatty acid synthesis in chloroplasts of hydrilla has been worked out.

(b) *Cardiolipins as transitory reserve of phosphatidyl residues*: In both plants and microbial systems, a close relationship between the fatty acid composition of cardiolipins and phosphatidic acid has been established. It is proposed that cardiolipins represent a transitory storage of phosphatidyl residues, whose level fluctuates in response to the metabolic needs of the system.

### Tea Biochemistry and Tea Technology

Contrary to the established view that the major biochemical changes taking place during the fermentation of tea were confined to the oxidation of 1-

epigallocatechin and its gallate only, it has been established that in addition 1-epicatechin gallate was also involved in this process. This finding revolutionized the approach to the structure of theaflavin and theaflavin gallate, the two important components of tea quality.

Subsequently, it was shown that 1-ECGG/1-ECG ratio remained constant under diverse cultural and environmental conditions and depended solely on the nature and origin of the leaf, suggesting that this ratio is genetically controlled. Based on this finding, it was suggested that it was possible to distinguish clones and cultivated varieties of tea plant on the basis of differences in their polyphenolic make-up. The loss in (-) epigallocatechin gallate has been used to calculate the extent of cell distortion increased by the leaf during rolling and cutting in different types of manufacturing machinery.

### Ruminant Biochemistry

It was shown that throughout the growth of the fodder crops, bajra, jowar and napier, fluctuations in the content of lignin, cellulose and hemicellulose follow a similar trend, indicating that they may be components of a common matrix in the cell wall.

It was established that hemicellulose of the lignin of leaf and stem was more efficiently utilized than cellulose. Cellulose digestion was negatively better correlated with the methoxyl content of the lignin of the plant than with either lignin or its methoxyl content.

The combined contents of lignin and tannins were better inversely correlated with digestibility than either lignin or tannin content alone.



Delignification of plant material improved cellulose digestion; hemicellulose chemically separated from cellulose was markedly more digestible than when in combination with it.

**Lipid metabolism in buffalo:** Digestibilities of saturated fatty acids increased with decrease in their chain length and melting points. Biohydrogenation of dietary 18:1 and 18:2 acids was almost complete after passage through the gut. The addition of coconut oil, ricebran oil or cottonseed oil at 6% level to the low fat diet fed to milch buffaloes increased the total cholesterol, total lipids and phospholipids in the blood serum. Cholesterol esters were the major serum lipid component, while phosphatidyl choline was the major phospholipid. The triglyceride content of serum lipids was depressed by cottonseed oil diet and not by other diets. Addition of unsaturated C<sub>18</sub> fatty acids to a low fat diet depressed the lecithin and lysolecithin contents and increased the sphingomyellin content, while addition of 12:0 and 14:0 acids had the opposite effect. Fatty acid composition of cholesterol esters was influenced by the type of fatty acids added to the low fat diet.

**Mammary gland metabolism in buffalo:** Under *in vitro* conditions, the buffalo mammary gland slices utilized 2-<sup>14</sup>C-acetate for biosynthesis of fatty acids and cholesterol, whereas U-<sup>14</sup>C-glucose was used for fatty acid and alpha-glycerol moiety. The hormones insulin, prolactin, insulin + corticosterone and insulin + prolactin + corticosterone increased the incorporation of 2-<sup>14</sup>C acetate into fatty acids and total lipids in buffalo mammary gland slices. Fatty acid synthetase complex (FAS) from lactating buffalo

mammary glands was isolated and characterized.

**Erucic acid metabolism:** It was shown that in rats, the oxidation of 1-<sup>14</sup>C-erucic acid was lower than that of 1-<sup>14</sup>C-oleic acid. There was little accumulation of lipids in the heart after 120 days of feeding mustard oil. Oxidation of 1-<sup>14</sup>C-erucic acid enhanced in the liver, heart and skeletal muscles of rats conditioned to the mustard oil diet supplying erucic acid. The heart of rats getting mustard oil at 10% and 15% levels for 2 days showed accumulation of lipids both by histological and biochemical examination.

**Analytical biochemistry:** A unique colorimetric method for the quantitative determination of phospholipids that does not involve the acid digestion of lipid has been reported.

### Selected Publications

1. Srinivasan M & Bhatia I S, The carbohydrates of *Agave vera* Cruz, *Biochem J*, **55** (1953) 286.
2. Srinivasan M & Bhatia I S, Carbohydrates of *Agave vera* Cruz. II. Distribution in the stem and poly, *Biochem J*, **56** (1954) 256.
3. Bhatia I S, Satyanarayana M N & Srinivasan M, Transfructosidase from *Agave vera* Cruz, *Biochem J*, **61** (1955) 171.
4. Randhir Singh & Bhatia I S, Isolation and characterization of fructosyl transferase from chicory roots, *Phytochemistry*, **10** (1971) 495-502.
5. Bhatia I S & Nandra K S, Studies on fructosyl transferase from *Agave americana*, *Phytochemistry*, **18** (1979) 923-27.
6. Bhatia I S, Gumber S C & Rangil Singh, Metabolism of free sugars in relation to starch synthesis in the developing *Sorghum vulgare* grain, *Physiol Plant*, **49** (1980) 248-54.
7. Nandra K S & Bhatia I S, *In vivo* biosynthesis of glucofructosans in *Agave americana*, *Phytochemistry*, **19** (5) (1980) 965.
8. Gupta A K & Bhatia I S, Glucofructosan biosynthesis in *Fusarium oxysporum*, *Phytochemistry*, **19** (12) (1980) 2557-63.

9. Bhatia I S, Manoharjit Kaur & Rangil Singh, Changes in carbohydrates during growth and development of cheena (*Panicum millaceum* Linn), *J Sci Fd Agric*, **25** (1974) 781-90.
10. Bhatia I S & Ullah M R, Phenolic metabolism of tea shoots, *Nature, Lond*, **103** (1962) 4816.
11. Bhatia I S & Ullah M R, Quantitative changes in polyphenols during the processing of tea leaf and their relation to liquor characters, *J Sci Fd Agric*, **16** (1965) 408-16.
12. Bhatia I S, Ahuja K L & Sukhija P S, Changes in the activity of acetyl CoA carboxylase in germinating and ripening sunflower seeds, *Physiol Plant*, **44** (3) (1978) 141-44.
13. Bhatia I S, Ahuja K L & Sukhija P S, Fatty acid synthesis in Hydrilla chloroplasts, *Physiol Plant*, **47** (1979) 81-86.
14. Bhatia I S, Saxena A K & Ahuja S P, Effects of prolactin, insulin and corticosterone on the *in vitro* incorporation of 2-<sup>14</sup>C-acetate and U-<sup>14</sup>C-glucose into lipids by mammary gland slices from a lactating buffalo, *Zbl Vet Med A*, **26** (1979) 306-17.
15. Rajeja R K, Charanjit Kaur, Ajit Singh & Bhatia I S, New colorimetric method for the quantitative estimation of phospholipids without acid digestion, *J Lipid Res*, **14** (1973) 695-97.



## Bishwajit Choudhury

The major contributions of Choudhury are discussed below in brief.

(1) Twenty-eight vegetable cultivars and hybrids were released to the farmers as a result of the work done under projects with which Choudhury was directly involved.

*Tomato*—Pusa 120 (the first root-knot nematode resistant variety released in India); Pusa Gaurav—a recent release—high yielding, suitable for processing; Pusa Sheetal—a cold-set tomato.

*Brinjal*—Pusa Kranti (high yielding, attractive deep purple); Pusa Anmol ( $F_1$  hybrid gives 100% higher yield than Pusa Purple Long); Pusa Bhairab—a phomopsis rot resistant high yielding variety.

*Bottle gourd*—Pusa Meghdoot ( $F_1$  hybrid) and Pusa Manjari ( $F_1$  hybrid), Sl. 2, Sl. 11; Pusa Naveen—an early high yielding variety.

*Radish*—Pusa Desi and Pusa Chetki (the only variety being grown during hot summer, April to August, in the plains of India).

*Bittergourd*—Pusa Domousmi; Pusa Vishes.

*Palak*—Pusa Jyoti.

*Muskmelon*—Pusa Sharbati and Pusa Madhuras.

*Watermelon*—Sugarbaby and Pusa Badana (Seedless), Pusa Rasaal and Tetra-2.

*Peas*—Arkel (most popular early, attractive, ready for picking in 55 days) and Meteor.

*Onion*—Pusa White Flat and Pusa White Globe (both suitable for dehydration).

*Pumpkin*—Pusa Vishwas.

The above cultivars and hybrids have become very popular with vegetable growers throughout the country. Most of them have been accepted by the National Seeds Corporation and the seeds are being multiplied by them as well as by private seed firms.

(2) Sources of resistance to the under-mentioned diseases, insect pests and root-knot nematodes have been identified:

*Tomato*—Tobacco mosaic virus, Leaf curl virus, *Fusarium* wilt and root-knot nematodes.

*Brinjal*—*Phomopsis vexans*. Little leaf diseases and root-knot nematodes.

*Muskmelon*—Powdery mildew and red pumpkin beetle.

*Catexmelon*—Red pumpkin beetle.

*Bottlegourd*—Red pumpkin beetle.

*Peas*—*Fusarium* wilt.

The pattern of inheritance of resistance has been worked out and the resistance is being incorporated in the cultivated varieties. Some are at advanced stage of testing before release, particularly a brinjal line resistant to *Phomopsis* rot and a tomato line resistant to leaf curl virus.

(3) Choudhury demonstrated for the first time in India that chemical sex modification is possible in monoecious cucurbits under high temperature and long day conditions. The action of gibberellic acid at low concentration in increasing the number of female flowers in crops like cucumber, watermelon and bottle gourd was reported. The efficacy of cheap chemicals like boron and molybdenum at a very low concentration of 3 ppm in increasing the number of female flowers and increasing the yield in monoecious cucurbits by 50-80% was reported for the first time. These results have now been tried on a large scale in farmers' fields and significant increases in yield have been achieved in several cucurbits. The mode of action of these chemicals has been studied using radioactive chemicals and also through anatomical and chemical studies.

(4) The practical utilization of  $F_1$  hybrid seeds in vegetable crops has been demonstrated and advocated for increasing production. The use of male sterile lines in tomatoes and gynoecious lines in cucumber has been demonstrated. Large scale commercial production of  $F_1$  seeds in bottle gourd has been made

possible and adopted by seed nurseries. Good combiners in bitter gourd and pumpkin have been identified.

(5) It was demonstrated that by seed treatments and whole plant spray, which can be adopted practically on a large scale, the yield of vegetable crops like tomato, brinjal and cowpea can be increased significantly. The chemicals which were cheap, easily available and effective are 2,4-D at 2.5 ppm for tomato and brinjal and maleic hydrazide at 15 ppm for cowpea.

(6) Selection of a suitable cultivar, N-53, as Kharif onion, has brought about a revolution in onion cultivation in the plains of northern India.

(7) It has been successfully demonstrated that the opium, morphine and codein contents of poppy can be increased by the use of  $F_1$  combinations and also by other breeding techniques.

(8) The dormancy in the freshly harvested corms of gladiolus can now be reduced from three months to about a month by a combination of treatments of chemicals and temperature.

Choudhury has published more than 220 scientific and other papers and has guided 53 postgraduate students (23 for MSc and 30 for PhD) in their research.

For his research contributions, Choudhury has received a number of awards, including the Rafi Ahmed Kidwai Prize, Borlaug Award, AISMAN Award and FICCI Award.

### Selected Publications

1. Choudhury B, Hybridisation between *Lycopersicon esculentum* and *L. peruvianum*, *Indian J Hort*, **16** (1959) 102-7.



2. Choudhury B & Phatak S C, Sex expression and sex ratio in cucumber as affected by plant regulator sprays, *Indian J Hort*, **16** (1959) 233-35
3. Choudhury B & Babel Y S, Studies on sex expression, sex ratio and fruit set as affected by plant regulator sprays in bottle gourd, *Horticult Sci*, **1** (1969) 61-71.
4. Choudhury B & Babel Y S, Sex modification by chemicals in bottle gourd (*Lagenaria siceraria*), *Sci & Cult*, **35** (1969) 321-22.
5. Tewari R, Choudhury B & Raychoudhari S P, Tobacco mosaic virus resistance in *Lycopersicon*, *Indian Phytopath*, **25** (1972) 392-400.
6. Singh B & Choudhury B, The chemical characteristics of tomato cultivars resistant to root-knot nematodes (*Meloidogyne* spp.), *Nematologica*, **19** (1973) 411-16.
7. Choudhury B, Vegetables, their problems and prospects in India, *Presidential Address, Section of Agricultural Sciences, 61st Session, Indian Science Congress* (1974) 31-54.
8. Chakrabarti A K & Choudhury B, Breeding brinjal resistant to leaf disease, *Proc Indian natn Sci Acad*, **41B** (1975) 379-85.
9. Ram Phal & Choudhury B, Screening of pea resistance to *Fusarium* wilt, *Indian J agric Sci*, **48** (1978) 407-10.
10. Kesavan V & Choudhury B, Screening for resistance to *Fusarium* wilt of tomato, *SABRAO J*, (1978) 81-83.
11. Sivakami N, Choudhury B & Murty B R, Genetic basis of powdery mildew resistance in muskmelon, *Scientia Horticulturae*, **10** (1979) 167-76.
12. Rao M Rama & Choudhury B, Studies on inheritance of processing qualities of tomato (*Lycopersicon esculentum*), *Vegetable Sci*, **6** (1979) 113-25.
13. Joshi G C & Choudhury B, Screening of *Lycopersicon* and *Solanum* species for resistance to leaf curl virus, *Vegetable Sci*, **8** (1981) 45-50.
14. Singh R K, Choudhury B & Sivakami N, Uptake and translocation of gibberellic acid in cucumber, *J nucl agric Biol*, **13** (1984) 1-4.
15. Choudhury B, Vegetables and quality of life in the year 2000, *10th Anniversary Monograph Ser*, Asian Vegetables Research and Development Centre, 1984.

## Rajat De

The contributions of De have been mostly in the field of dryland agronomy, including nutrient use and water conservation. In earlier days, he was involved with fertilizer use in irrigated crops at the Indian Agricultural Research Institute, New Delhi; agronomy of potatoes at the Central Potato Research Institute, Simla; and fertilizer use in sugarcane at the Sugarcane Research Station, Jullundur in Punjab. He has a total experience of 35 years in the field of crop production research and teaching at post-graduate level.

Traditionally, only one crop is grown in a year in the semi-arid rainfed tracts of north west India, where the monsoon rains last 2-2.5 months. The rains in the post-monsoon season are meagre and mostly uncertain. Under these conditions, it has been shown that by growing a short duration crop of grain or fodder legume and harvesting it within 60 days, it is possible to conserve the tail-end monsoon rains to recharge the profile and thus raise a post-monsoon crop. The legumes grown during the monsoon period help enrich the soil N status, thus augmenting the yield of the winter season crop.

Another possibility of double cropping in the dryland areas is through the use of straw mulch in the monsoon season crops, which helps to prevent the surface

evaporation loss of water, retaining sufficient moisture in the profile to establish a second crop. This "minimum tillage" practice is a great boon to the dryland farmer who can raise the biomass *in situ* for mulching purposes. In some parts of the country, as in the foothills of the Himalayas, many farmers have adopted this practice extensively.

Wheat is the dominant crop during the *rabi* season in north and north-western India, but its yields in dryland situations are abysmally low (about 800 kg/ha). This is because farmers sow their crop in mid-October, when the atmospheric temperatures are still high. Experiments conducted for over a decade have shown that by delaying the sowing to the first week of November, the yielding ability of wheat can be raised 3 or 4 times. The temperature relationships at critical phenological stages of the wheat plant are clearly established by this study.

Because of aberrant weather in rainfed situations, yield stability of most crops is affected adversely. Experimental results have, however, indicated that crop production can be stabilized if mixtures of two crop species, which tap different soil strata for nutrient and water resources, are grown rather than a single crop. Using labelled N(<sup>15</sup>N), it has been shown that when a mixture of legumes and non-



legumes is grown, the non-legume is benefited concurrently from the legume. The advantage to sorghum when grown in association with groundnut was 10-15% in terms of seed yield *vis-à-vis* a sole sorghum crop. The land utilization efficiency of the mixture was 26% more than that for either of the single crops. This work has helped in the understanding of the behavioral pattern of crops when grown in mixture and has given cogent proof to show how cereals benefit from their association with grain legumes.

With the advent of the high yielding varieties of crop plants, the use of higher levels of fertilizers and management practices was advocated. Since fertilizers are relatively high-cost inputs and many nutrient elements when once applied to the soil can benefit a succession of crops, the question arose whether all the crops grown in a sequence be given the recommended rates of fertilizers. Long-term experiments conducted for more than a decade have established that while a mobile element like N needs to be applied at optimum rates to each crop grown in succession, it will be sufficient to use half the recommended rate of P for each crop. It was also shown that if legumes precede cereals, the fertilizer N application to the cereal crop could be reduced by 25-30 kg N/ha even if the legumes are grown for seed purposes. This has considerable economic implication for the farmers.

These research findings have found place in world literature in the form of contributions in publications brought out by Gerald Duckworth (London), Academic Press (NY) and the Food and Agricultural Organization (Rome). The number of research articles published exceeds 175.

## Selected Publications

1. De Rajat, Agronomy of dwarf wheats, in *Change in Agriculture* (Gerald Duckworth, London), 1969.
2. De Rajat, Agronomic management of crops and cropping systems, in *Advances in food producing systems for arid and semi-arid lands* (Academic Press, New York) 1979.
3. De Rajat, Efficient fertilizer use in summer rainfed situations, in *Fertilizer use in problem areas* (FAO, Rome) 1986.
4. Patil B B & De Rajat, Influence of antitranspirants on rapeseed (*Brassica campestris*) plants under water stressed and non-stressed conditions, *Pl Physiol*, **57** (1976) 941-43.
5. De Rajat, Gupta R S, Singh S P, Pal Mahendra, Singh S N, Sharma R N & Kaushik S K, Interplanting maize, sorghum and pearl-millet with short duration grain legumes, *Indian J agric Sci*, **48**(3) (1978) 132-37.
6. De Rajat & Giri Gajendra, Effect of mulching and kaolin foliar spray on mung (*Vigna radiata*) and rapeseed (*Brassica campestris*) double cropping system under rainfed conditions, *J agric Sci, Camb*, **91** (1978) 191-97.
7. Patil B B & De Rajat, Studies on the effect of nitrogen fertilizer, row spacing and use of antitranspirants on rapeseed (*Brassica campestris*) grown under dryland conditions, *J agric Sci, Camb*, **91** (1978) 257-64.
8. Lal B B, De Rajat & Singh R K, Legume contribution to fertilizer economy in legume-cereal rotations, *Indian J agric Sci*, **48**(7) (1978) 419-24.
9. Agarwal S K & De Rajat, Effect of rates of nitrogen, mulching and antitranspirants on nutrient uptake of barley varieties grown under rainfed conditions, *Indian J Agron*, **24**(1) (1979) 66-71.
10. Agarwal S K & De Rajat, Effect of nitrogen rates, mulching and antitranspirants on consumptive use of water and water use efficiency of barley (*Hordeum vulgare* L.) varieties grown under dryland conditions, *J agric Sci, Camb*, **92**(2) 263-69.
11. Sharma S K & De Rajat, Effect of water regimes, levels of nitrogen and methods of nitrogen application on grain yield, protein percentage and nitrogen uptake in rice, *Il Risi*, **28** (1979) 45-52.

12. Mohta N K & De Rajat, Intercropping maize and sorghum with soya beans, *J agric Sci, Camb*, **95** (1980) 177-82.
13. Giri C & De Rajat, Short-season fodder legume effects on the grain yield and nitrogen economy of barley under dryland conditions, *J agric Sci, Camb*, **96** (1981) 457-61.
14. Singh R K & De Rajat, Long-term effect of N and P fertilization on a pearl millet wheat cropping system, *Fert Res*, **3** (1982) 127-39.
15. De Rajat, Bheemiah G, Ramsheshiah K & Yogeswara Rao Y, Effect of mulches and antitranspirants on the grain yield of sorghum grown under limited irrigations on a deep vertisol, *J agric Sci, Camb*, **100** (1982) 159-62.
16. De Rajat, Bhujanga Rao D V S, Yogeswara Rao Y, Giri Rao L G & Ikramullah M, Modification of irrigation requirement of wheat through mulching and foliar application of transpiration suppressants, *Irrig Sci*, **4** (1983) 215-23.
17. Bandyopadhyay Sanjoy K & De Rajat, N relationship in a legume-nonlegume association grown in an intercropping system, *Fert Res* (1985) in press.
18. Bandyopadhyay S K & De Rajat, Studies on sorghum based intercropping system with legumes. I. Plant growth and seed yield, 2.N relationships and quantification of residual effects, *J agric Sci, Camb*, **107** (1986).



## G B Deodikar\*

It is normally considered proper to restrict to one's own specialization in a conventional discipline of science without trespassing into other subject fields. However, some individuals develop a strange habit of pursuing their impulsive curiosities about any facet of nature that appeals to them and triggers their imagination into running riot. Such trespassers may cross the limits of even permissible interdisciplinary research, thus inviting pious opposition from relevant specialists. To this category belongs the present case of a free-lance career in science.

After graduating with chemistry, botany and zoology from the Fergusson College, Poona, in 1937, I simultaneously studied law at the Law College, Poona and started postgraduate research in genetics at the College of Agriculture, Poona, getting LLB in 1941 and PhD (Bombay University) in 1942 for my research thesis on "Cytogenetic survey of the genus *Commelina* as distributed in India, Burma and Ceylon". These studies revealed an interesting mechanism of speciation leading to the evolution of primary and secondary chromosomal balance with characteristic trends in their karyogeographical distribution in the area covered<sup>1</sup>.

During 1942-1950, I served in the State Agricultural Department on research posts as an assistant chemist, cytologist, geneticist and plant-breeder in research projects for evolving improved varieties of rice, millets, cereals, cotton, pulses, oilseeds and other crops. During this period, I developed a collection of over 1500 wild species related to various cultivated crops, reported findings on wild ancestors of maize and sorghum<sup>2</sup>, indicated the utility of wild African diploid cotton, *Gossypium anomalum*, as a bridging species for inter-specific transference of desired genes and succeeded in addition or substitution of individual chromosomes of the wild African *G. anomalum* to the genome of cultivated American tetraploid cottons (*G. hirsutum*). Such programmes, then, were very rare in other countries and this was the first report of its kind in India<sup>3</sup>.

It was reported with conclusive proof that the most important Indian pulse crop, *Cajanus indicus* Spreng., has evolved from closely related wild ancestral species of *Atylosia*<sup>4</sup>. On the basis of this report, (*Cajanus* × *Atylosia*) hybridization is being employed extensively in India and other tropical countries for improving this pulse crop.

I resigned government service in 1950 and was soon invited by the State Village

---

MACS Research Institute, Maharashtra Association for the Cultivation of Science, Pune-411004.

\*Since deceased.

Industries Board to organize a research centre for the bee-keeping industry in 1951 at Mahabaleshwar. This subject was mostly handled by agricultural entomologists in various states with their emphasis restricted to entomological aspects. The new Bee-keeping Research Centre at Mahabaleshwar organized comprehensive inter-disciplinary programmes, which besides entomology included studies on local bee-floras, floral calendars of the bee plants, bee botany, bee palynology for identification of floral sources of honeys, bee-pollination of crop plants, bee genetics, breeding of better bee-strains, chemistry of bee products, bee management techniques, formulation of ISI standards for bee-keeping equipment and bee products and other disciplines relevant to Indian species of honey bees and bee-keeping.

With the cooperation of a small number of highly devoted younger colleagues, including C V Thakar, R P Phadke, Salvi and others, this programme soon started yielding results of theoretical and practical significance. The first Indian reports on bee genetics<sup>5</sup>, bee breeding<sup>6</sup>, bee palynology<sup>7</sup> and design of experiments for estimating increase in crop yields due to their bee pollination<sup>8</sup> were published by our group. As a result of these achievements, this modest "Bee-keeping Research Centre" was upgraded as an "Apicultural Laboratory" at Mahabaleshwar in 1956 by the Village Industries Board of the Maharashtra State.

With further expansion of its research programmes, scientific achievements of this laboratory assumed a wider significance for the country as a whole. Therefore, the All-India Village Industries Commission took it over in 1961 and

upgraded it further as the Central Bee-keeping Research Institute (CBRI) located at Poona with its own regional laboratories and field-observation stations at various places in India. The National Commission on Agriculture has recommended recognition of CBRI as the "National Institute" for bee-keeping research in India. The Indian Council of Agricultural Research has assigned to CBRI the function of coordinating bee-keeping research in India. CBRI is the only institute of its kind in Asia and the tropical belt. Many developing countries send their young scientists here for training in research methodology in tropical apiculture. I was associated with this project first as its Hon. Founder Director and then as Scientific Adviser from 1952 to 1977.

Presence of mulberry trees and castor plants around the Apicultural Laboratory at Mahabaleshwar stimulated my interest in genetics and breeding of Indian silkworms and their food plants. A beginning was made in a corner of the Apicultural Laboratory itself in 1956. This led to our first Indian reports on chromosomal constitutions of castor silkworms (*Attacus ricini*)<sup>9</sup>, Muga silkworms of Assam (*Antheraea assamensis*)<sup>10</sup>, and the wild related species (*Actias selene*)<sup>11</sup> with reelable cocoons.

An unconventional attempt was made to expose unfertilized eggs of a homozygous strain of mulberry silkworm (*Bombyx mori*) to the spermatid fluid of castor silkworm (*Attacus ricini*), hoping to get their inter-generic hybrids by such external fertilization of eggs. Some of the exposed eggs hatched into abnormal larvae. Their chromosomal studies revealed that it is not a case of hybridization, but of



parthenogenesis. Though the female strain was homozygous, the parthenogenetic larvae on inbreeding gave wide-ranging variations in unexpected characters, implying possible transfer of genetic elements from sperms without fertilization. In view of such unexpected results, these experiments were repeated thrice confirming similar findings. These results were obtained around 1960 before the discovery of reverse transcriptase and the recent techniques of gene transfers. Sophisticated facilities, such as tracer techniques with labelled  $^{32}\text{P}$  were not available to us in our simple cottage laboratory in forests. Therefore, these findings were reported<sup>12</sup> as such without further exploration of theoretical aspects. But we utilized the wide-ranging variations in their inbred progenies for genetic selection of superior fixed strains combining higher silk yields, disease tolerance and resistance to inbreeding depression<sup>13</sup>.

A parallel programme of breeding mulberry food plants has yielded types combining higher leaf yields with tolerance to drought in low rainfall areas. A small farmer can plant about an acre under these mulberry varieties, utilize their leaf for rearing improved strains of silkworms after a short training and earn about Rs 500 per month as a part-time self-employment. These results have introduced a new rural industry in Maharashtra where sericulture was unknown. Therefore, the Village Industries Board (MS) provided special grants and facilities for establishing the Sericultural Laboratory at Panchgani in Mahabaleshwar Hills in 1958.

While organizing research projects on Indian honeybees and silkworms at

Mahabaleshwar and Panchgani as above, I simultaneously worked at the Research Institute, Maharashtra Association for the Cultivation of Science (MACS) at Poona, first as the Hon. Cytogeneticist (1953-1960), then as the Hon. Director (1960-1965) and finally as Director (1965-1980). Applied research on bee-keeping and sericultural industries was done at Mahabaleshwar and Panchgani and purely academic basic research on bees and silkworms was done at the MACS through inter-institutional collaboration. As the President of the Indian Society of Genetics for 1975, I delivered the annual presidential address<sup>14</sup> on "Genetics and breeding of some beneficial insects and their food-plants". On the basis of distribution of endemic species of honeybees, silkworms and their food plants along the tropical belt, the possible origin of flowering plants prior to the Continental Drift was indicated<sup>15</sup>. Besides guiding a number of PhD students, I also taught genetics to MSc students as a Visiting Hon. Professor at the Poona University during 1954-1977. Research projects were developed at the MACS for genetics and breeding of improved strains of wheats, soyabeans, grapes and other crops released to farmers.

I was thus associated with the foundational work of the Central Bee-keeping Research Institute at Poona and the Sericultural Laboratory at Panchgai, as also with the developmental work of the MACS Research Institute at Poona. It is a pleasure to see such large number of senior and young scientists working at these three institutions contributing to the progress of science.

After retirement from the post of Director, MACS, I now continue a few of

my scientific interests as Professor Emeritus (Agharkar Chair) there. Besides a project on breeding better strains of perennial cottons suited as hedge-rows along farm bunds, I have now revived my earlier<sup>16</sup> interests in social obligations of science by publishing serial papers<sup>17</sup> on "Infrastructural imperatives for Indian socio-economic reconstruction".

In the course of my routine cytological laboratory work, I first observed around 1940 peculiar sequential patterns of discontinuous evaporations of the solvent phase of fluid drops of certain biological stain, leaving behind solute phase sediments on glass slides in concentric rings resembling the solar system with planets. This stimulated my spare time interests in highly speculative astronomical theories regarding the evolution of stars and origin of the solar system. These spare time interests resulted in four serial papers<sup>18</sup> on "Fluid-drops as cosmological models".

### Selected Publications

1. Deodikar G B & Kumar L S S, Gross-over suppression and linear differentiation of homologues as a genetic isolation mechanism leading to secondary chromosomal balance in the genus *Commelina*, *Indian J Genet (Mendel Symp Vol)*, **26(A)** (1966) 274-83.
2. Deodikar G B, *Sorghum versicolor*, a species highly resistant to striga, *Curr Sci*, **20** (1961) 135.
3. Deodikar G B, Cytogenetic studies on crosses of *Gossypium anomalum* with cultivated cottons. II. Substitution and addition of anomalum chromosomes to the genome of cultivated tetraploid cottons, *Indian J agric Sci*, **20**, 399-414.
4. Deodikar G B & Thakar C V, Cyto-taxonomic evidence for the affinity between *Cajanus indicus* Spreng. and certain erect species of *Atylosia*, *Proc Indian Acad Sci*, **43(B)** (1956) 37-43.
5. Deodikar G B, Thakar C V & Tonapi K V, Evolution of the genus *Apis* and its bearing on breeding improved strains of Indian honeybees, *Proc 17th International Bee-keeping Congress, Bologna-Rome, 1958*, 245-50.
6. Deodikar G B & Thakar C V, Cytogenetics of Indian honeybees and its bearing on taxonomic and breeding problems, *Indian J Genet (Mendel Symp Vol)*, **26(A)** (1966) 386-93.
7. Deodikar G B, *Melito-palynology*, in *Advances in palynology*, edited by P K K Nayar (National Botanic Gardens, Lucknow) 1964, 404-19.
8. Deodikar G B, A design for experiments on bee-pollination of crops, *Apiacta*, **5** (1970) 6-8 (in English, French, Russian, German and Spanish).
9. Deodikar G B & Thakar C V, Cytogenetic studies in Indian silkworms: I. Spermatogenesis in castor silkworms (*Attacus ricinii*), *Curr Sci*, **27** (1958) 457-58.
10. Deodikar G B, Chaudhari S N, Bhuyan B N & Kshirsagar K K, Cytogenetic studies in Indian silkworms: II. Chromosome number in *Antheraca assamensis*. Westwood, *Curr Sci*, **39** (1962) 247-48.
11. Deodikar G B, Kshirsagar K K & Kamate I A, Chromosome number in *Actias selene* Hubner, a wild silkworm with reelable cocoons, *Indian J Genet*, **29** (1969) 120-30.
12. Deodikar G B, Kamate I A & Kshirsagar K K, A possible case of genetic transformation in *Bombyx mori* L. and its application for breeding improved strains of silkworms, *Indian J Genet (SABRAO Congress)*, **34A** (1973) 263-66.
13. Deodikar G B, Kamate I A & Kshirsagar K K, New strains of mulberry silkworms (*Bombyx mori* L.) by induced anomalous parthenogenesis, *Indian J Seric*, **26** (1977) 43-48.
14. Deodikar G B, Genetics and breeding of some beneficial insects and their food plants, *Presidential Address, Indian Society of Genetics*, *Indian J Genet*, **35** (1975) 319-26.
15. Deodikar G B, Possible origin and diversification of angiosperms prior to Continental Drift, *Recent Res Geol*, **4** (1978) 473-81.
16. Deodikar G B, A design for the layout of Indian transport & communication system (Indian Society of Agricultural Economics, Bombay) 1949.
17. Deodikar G B, Infra-structural imperatives for Indian socio-economic reconstruction: Soc & Sci, Part I—5(1) (1982) 56; Part II—5(2) (1982) 21; Part III—6(2) (1983) 40.
18. Deodikar G B, Fluid-drops as cosmological models, *Speculat Sci Technol*, Part I—1(3) (1978) 253; Part II—1(4) (1978) 359; Part III—3(1) (1980) 49; Part IV—5(3) (1982) 317.



## P D Dogra

The genetic diversity found in widely distributed tree species forms the basis for germplasm selection and tree improvement work. India is rich in indigenous tree species. A survey and assessment of intraspecific variation present in some Indian tree species was carried out within their distributional ranges.

Reproductive biology was also studied for germplasm lifting, experimental identification, multiplication and breeding. The studies were carried out for use in forestry and forest genetics<sup>1,2</sup> and for botanical information required to deal with problems of taxonomy and evolution<sup>3,4</sup>.

### (1) Natural Variability and Genetic Improvement

*Pinus* : Studies on natural variation were conducted under a survey of *P. gerardiana* forests in the Upper Sutlej valley<sup>5</sup>. Four distinct forest types with specific populations were identified. In each population, tree to tree variation was also shown to be present. This work shows that selection at tree and provenance levels opens up the possibility of genetic improvement for edible pine seed. Selections can be used for the establishment of seed orchards with stock scion grafts for the production of highly prized chilgoza seed.

*Pinus wallichiana* is one of the important timber trees of Northwestern Himalayas. It is rich in high quality oleoresins. It crosses with a number of elite species of the white pine group giving improved hybrids<sup>6</sup>. A survey of intraspecific natural variability was carried out in its natural distributional range in the Northwestern Himalayas. Observations on blue pine forests of Eastern Himalayas were included. Seven altitudinal provenance types were defined<sup>6</sup>. Four were shown to be adapted to the outer moist and inner dry Northwestern Himalayas, and three to the outer wet, middle moist and inner dry Eastern Himalayas.

Genetic variation can be experimentally separated from geographical variation by progeny tests conducted under uniform environment. Seed provenances were collected from widely separated regions from different altitudes and latitudes of Northwestern Himalayas and single tree and provenance progeny studied in a large seed origin trial laid out in 1970 in collaboration with Prof. H B Kriebel at Wooster, Ohio. The results are useful for genetic improvement of the Himalayan blue pine.

Selection of superior trees was made for seed collection and conservation of genetic diversity. Seed production can be achieved

---

Assistant Director, National Botanical Research Institute (CSIR), Lucknow-226001; Residence : B-73, Sector C, Mahanagar, Lucknow-226005.

through seed orchard technology. Flowering and seeding characteristics are, therefore, important. Field observations were made for six years to study variability in the biology of flowering in different *P. wallichiana* provenances of Northwestern Himalayas<sup>7</sup>. A weak reproductive barrier is shown to exist between the provenances growing at lower and higher altitudes of both moist and dry zones, but a strong reproductive barrier blocking gene flow is functional between the moist and the dry arid zone populations in Himachal and Uttar Pradesh.

Oleoresin yield and quality is an inherited character in pines. That *P. roxburghii*, *P. wallichiana* and *P. kesiya* have a vast potential was shown from a data survey made on oleoresin yielding pines of India<sup>8-10</sup>. High yield and superior turpentine quality can be achieved through selection from natural variation and breeding of Indian pines.

*Genetic tree improvement potential of Indian species*: The genetic potential of both broad leaved and conifer trees of India was assessed from field observation, experimentation and data survey<sup>10,12</sup>. It was shown that the greatest improvement potential exists in the selection from wild gene resources. A practical methodology to utilize forest genetics in improvement of Indian forests was laid out for use by Indian foresters<sup>11,12</sup>. Germplasm selection from wild forests and conservation *in situ* and *ex situ* are practical steps that require immediate attention.

*Moringa oleifera* and *M. concanensis* are two fast growing tropical trees widely distributed in India. Data from experiments conducted at Lucknow show the presence of sufficient genetic variability in *M. oleifera* to make selection breeding a

profitable venture for its use for food, vegetables, fodder and medicine<sup>13</sup>. Interspecific hybrids between *M. oleifera* and *M. concanensis* were raised. The hybrids are superior to the parents, show a combination of useful characters and can be propagated by cuttings. Clones of *M. oleifera* were shown to be of two distinct groups, one flowering once and the other twice a year. The latter proved to be important for vegetable production and were termed as 'baramassi' types.

Extensive intraspecific variation occurred in size and taste of pods in *M. oleifera* and *M. concanensis*. Vegetable pods of selected clones of *M. oleifera* showed high nutritive contents. Selections of *M. oleifera* clones were, therefore, made from widely separated regions. Raised by cuttings, they were planted in randomized rows and replicated to form vegetable orchards raised under isolation. High yields of pod vegetables with good pod lengths, pulp content and sweetness were harvested. These pod vegetables showed clearcut improvement in nutritive contents<sup>10</sup>. *M. oleifera* can, therefore, be developed as a high quality vegetable producing tree.

## (2) Reproductive Biology

*Basic aspects*: Problems concerning pollination mechanisms<sup>5</sup>, apomixis and haploidy<sup>1,14</sup>, embryogeny and polyembryony in Pinaceae<sup>1,3,4</sup>, Taxodiaceae<sup>15</sup>, Cupressaceae<sup>16</sup>, and gymnosperms in general<sup>4</sup> were studied. The basal pattern of development of the conifer embryo was discussed and a proper terminology based on morphological concepts suggested<sup>1,3</sup>. The basal plan of embryogeny of conifers and of gymnosperms as a whole was analysed and evolutionary tendencies traced out<sup>4</sup>.



A monograph dealing with the embryology, breeding systems, pollination mechanisms and seed sterility in Cupressaceae discusses data on four species of *Cupressus*, four of *Juniperus* and one each of *Chamaecyparis* and *Thujaopsis*<sup>16</sup>. The genera studied are large and well distributed and represent three tribes of Cupressoideae. Data available on most mother genera were updated and compared. Different evolutionary tendencies were traced in both the subfamilies Cupressoideae and Callitroideae in which embryological characters were shown to be linked with taxonomy. Observations on breeding systems and seed sterility were added. The phenomenon of multiple male cell formation in *Cupressus* species was investigated and it was shown that multiple male cell formation is derived from a condition of double male cell formation. It was concluded that advanced and primitive characters of gymnosperm embryology need revision for use in taxonomical and evolutionary studies. New and reliable parameters were, therefore, laid out for advanced and primitive characteristics of reproductive biology of conifers and of gymnosperms in general<sup>4,16</sup>. Based on studies on Himalayan conifers of forestry value extending over 30 years, a book "*Reproductive Biology of Pinaceae—Basic and Applied Aspects*" is under preparation. It is based on observations made on *Pinus*, *Abies*, *Picea* and *Cedrus* species. Work on reproductive aspects of *Taxus*, *Cephalotaxus* and *Podocarpus* is also under completion and the embryological data on other gymnosperms including those on *Ginkgo* and *Cycads* is under study.

**Applied aspects:** The control and genetic manipulation of superior genetic

variability is achieved through judicious use of both the sexual (self- or cross-pollinated seed) and the asexual (vegetative propagation) reproductive systems. The problems of pollination, breeding systems, annual flower and seed production, seed ripening and dispersal, seed orchard establishment and hybridization work were discussed for conifers<sup>2</sup> and for broad leaved trees. In conifers, pollen grains and female pollen receiving devices have been described in relation to pollination mechanisms for use in breeding work<sup>5</sup>. The knowledge has been used to judge differences in periods of receptivity and pollen shed in different provenances of *P. wallichiana* in a study of reproductive barriers<sup>7</sup>. This is shown to initiate and maintain species diversity. Initiation of spontaneous haploidy is reported in some open pollinated trees of *Pinus wallichiana* and *P. nigra* var. *austriaca*<sup>1,10,14</sup>.

Seed sterility and disturbances in embryogeny were discussed in Pinaceae, with particular reference to seed testing and tree breeding work<sup>1</sup>. The use of X-ray radiography in detecting empty, undeveloped, abnormal and full seed in natural populations and in controlled pollination and hybridization work was standardized on the basis of embryo and endosperm studies. The work is of use in the control of seed quality and in screening of provenances, mother trees and hybrids for seed fertility. It will help in application of embryological knowledge in forest tree breeding. It has been shown that breeding systems of gymnosperms are simple and primitive in contrast to those of angiosperms. Most species show cross-pollination, but nothing prevents self-pollination, as gene controlled barriers do not appear to have evolved in

gymnosperms. Self-pollination leads to self-fertilization and embryo inception. In most conifers, embryo mortality is the mechanism that limits selfing. This was shown in a study of the embryology of self-pollinated material<sup>1,2</sup>. In the production of seed from natural stands and seed orchards, self-pollination and empty seed formation are potential problems.

Screening for reproductive adaptability of fast growing exotic tropical pines successful in Kumaon Himalayas was carried out by studying their reproductive biology for four consecutive years on fixed sites. The differences shown to occur between species are inherent, as site and environment remained constant for each year.

The above studies have shown that work on survey and control of genetic variability of Indian tree species deserves an organized effort. Natural vegetative propagation characteristics of Indian trees must receive special attention for selective genotype lifting, cloning and conserving germplasm for use in forestry and genetic tree improvement work. Grafting techniques need to be developed for the establishment of seed orchards. Most trees are propagated from seed and genetic control through sexual reproduction (seed) requires a good knowledge of reproductive biology of tree species.

### Selected Publications

1. Dogra P D, Seed sterility and disturbances in embryogeny in conifers with particular reference to seed testing and tree breeding in Pinaceae, *Stud Forest Suec (Stockh)*, **45** (1967) 1-97.
2. Dogra P D, Reproductive biology of conifers and its application in forestry and forest genetics, *Phytomorphology*, **33** (1983) 142-56.
3. Dogra P D, Morphology, development and nomenclature of conifer embryo, *Phytomorphology*, **28** (1978) 307-22.
4. Dogra P D, Embryogeny of gymnosperms and taxonomy—An assessment, in *Glimpses in plant research*, Vol 5, edited by P K K Nair (Vikas Publishing House, New Delhi) 1980, 114-28.
5. Dogra P D, Pollination mechanisms in gymnosperms, in *Advances in palynology*, edited by P K K Nair (National Botanic Gardens, Lucknow) 1964, 142-75.
6. Dogra P D, *Intrinsic quality growth-and adaptation-potential of Pinus wallichiana*, US Dep Agric Forest Service, Misc Publ No 1221, 1972, 163-78.
7. Dogra P D, Variability in biology in flowering in blue pine provenances of Northwestern Himalayas in relation to reproductive barriers and gene flow, in *Proc Flowering Physiology, IUFRO, WP, Reprod Processes*, edited by L Krugman and M Katsuta, 17, IUFRO, 1981, 8-16.
8. Dogra P D, Oleoresin yielding pines of India, in *Proc Sympine, Seminar on Pine Resin*, 1971, New Delhi (Camphor and Allied Products Ltd, Bombay) 1971.
9. Dogra P D, Variability and genetics of oleoresin yielding pines, *Plant Sci*, **5** (1973) 7-18.
10. Dogra P D, Some aspects of tree-genetics research in India with particular reference to conifers, in *Progress in plant research*, Vol 1, edited by T N Khoshoo and P K K Nair (Today and Tomorrow's Publishers, New Delhi) 1979, 101-34.
11. Dogra P D, Natural variability and improvement potential of Indian tree species, in *Woodpower—New perspectives on forest usage*, edited by J J Talbot and W Swanson (Intern Sci and Tech Inst Inc, Washington DC/Pergamon Press, New York) 1981, 59-80.
12. Dogra P D, Forest genetics research and application in Indian forestry, Pts I, II, *Indian For*, **107** (1981) 191-219; 263-88.
13. Dogra P D, Pal A & Tandon S, Studies on breeding systems in Moringa. 3. Fruit-seed-set and seed-germination in two flowering periods of one year of the baramassi *Moringa oleifera* and chromosome pairing in the F<sub>1</sub> hybrid from *M. oleifera* and *M. cancanensis*, *Incomp Newslett (Netherlands)*, **6** (1975) 46-61.
14. Dogra P D, Observations on *Abies pindrow* with a discussion on the question of occurrence of apomixis in gymnosperms, *Silvae Genetica*, **15** (1966) 11-20.



15. Dogra P D, Embryogeny of the Taxodiaceae, *Phytomorphology*, **16** (1966) 125-41.
16. Dogra P D, Embryology and seed sterility in Cupressaceae; Associate paper: Dogra and Tandon, 1984, Observations on the embryology of *Juniperus procera* Hochst, in *Glimpses of plant research*, edited by P K K Nair (Vikas Publishing House, New Delhi) 1984 1-126.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

# Madhav Gadgil

I was attracted to the study of biology through my interest in bird watching and nature conservation. However, aware that this approach to biology often ends up as nothing more than stamp collecting, I made it a point to study mathematics as a graduate student in biology at Harvard University. I was the first student at Harvard to receive a PhD in biology for a mathematical thesis. I followed this theoretical work with field studies designed to test some of my own theoretical predictions. My subsequent work, the bulk of which has been carried out at the Indian Institute of Science, has combined theoretical investigations with field studies. The field studies have always been related to the theoretical framework and have been rigorously quantitative. My interests have spanned evolutionary ecology, sociobiology, human ecology, resource management, nature conservation and ecodevelopment.

## Evolutionary Ecology

I have been interested in exploring the consequences of the assumption that natural selection would have moulded the ecological as well as behavioural attributes of any organism so as to maximize genetic fitness. I have looked at the evolution of mortality, fertility and dispersal rates and competitive, altruistic and spiteful behaviour from this point of view. One of

the models I have explored considered an organism as solving the problem of optimal allocation of resources at its disposal between maintenance, growth and reproduction at various life history stages, a paper which has become one of the citation classics<sup>1</sup>. This model suggested that the nature of functional relationship, whether concave or convex between resources allocated to reproduction, current fertility and residual reproductive value, would determine whether an organism breeds repeatedly or just once after a long vegetative period. This theoretical prediction was then confronted with an empirical test by comparing yuccas which breed repeatedly, and agaves which flower only once. The theoretical prediction was verified neatly<sup>2</sup>.

I have gone on to elaborate the theory further to explore the differences between gregariously and sporadically flowering bamboo species of India. Again, the theoretical expectations neatly fit the situation in the field<sup>3</sup>.

## Sociobiology

I have considered the evolution of behaviour in the same spirit as the evolution of ecological parameters. An investigation of the limits to the escalation of competitive devices suggested that one should expect in nature two strategies:

---

Professor, Centre for Theoretical Studies and Centre for Ecological Sciences, Indian Institute of Science, Bangalore-560012; Residence : 28, 11th Main, Malleswaram, Bangalore-560003.



one of altogether opting out of competition and the second of going to the limit at which the burden of investment in competition is so high that it annuls the advantage of an edge in competition. This was one of the first demonstrations of the existence of mixed strategies in game theoretic terms. It predicted the occurrence of two kinds of males in many animal species that exhibit strong male-male competition<sup>4</sup>. This novel prediction has since been verified by a number of studies on insects, fish, birds and mammals.

I also studied the role of group selection in the evolution of social behaviour demonstrating the possibility of evolution of spiteful behaviour<sup>5</sup>.

The game theoretic approach to the evolution of social behaviour led to the first formal treatment of population genetics within this framework<sup>6,7</sup>.

### Human Ecology

The Indian society is remarkable for the persistence of a number of endogamous groups of hunter-gatherers, pastorals, nomads and semi-nomads, exhibiting intriguing adaptations for human use of natural resources. I have joined forces with an anthropologist to investigate these adaptations. This work led to the discovery of a remarkable differentiation of ecological niches of sympatric caste groups dependent on natural living resources. Such niche diversification provides clues to the persistence of India's caste system as well as traditions of prudent use of resources over more than a thousand years<sup>8-10</sup>.

### Resource Management

Management of India's renewable resources of soil, plant and animal life has

become a question of increasing concern as years have rolled by. It has become apparent that we have managed these resources in a manner that cannot be sustained in the long run. There has, however, been little careful scientific investigation of these problems, and the debate is obfuscated by claims and counter-claims with little firm database. My intensive studies of management of bamboo resources of Karnataka and the more extensive studies of the management of forest resources in India as a whole are among the few careful investigations of this problem<sup>11,12</sup>. These have formed the basis of the Department of Environment's recommendations for a new forest policy for the country.

### Nature Conservation

While the management of resources has been on a nonsustainable basis, the conservation of nature too has been taken up quite unsystematically. A major necessity of the day is, therefore, to rationalize our conservation effort to secure proper representation of each of India's biological community types. I have joined hands with a biogeographer to provide such a careful analysis<sup>13</sup>. I have also prepared at the instance of the Government of India, the project document for the first-ever biosphere reserve proposed to be set up in the country.

### Ecodevelopment

Our policy-makers have come to accept increasingly the need to include considerations of careful husbanding of natural resources in our development planning. What this means in terms of actual policies is, however, a question to which sound scientific inputs are very

essential. Much of my applied scientific work has attempted to generate this. It has been the thrust of my work on Western Ghats for which I was awarded the National Environment Fellowship for 1979-81. These ideas were summarized in a report to the Tiwari Committee set up to consider the issue. My report formed a basis on which the Government of India established the Department of Environment<sup>14,15</sup>.

I am currently involved in translating these ideas on ground through field experiments involving the local population in the district of Uttara Kannada.

It has thus been for me an exciting eleven years of basic and applied research at the Indian Institute of Science, and I hope to build a school of world class in this area in the coming years with the establishment of a Centre for Ecological Sciences.

### Selected Publications

1. Gadgil M & Bossert W H, Life historical consequences of natural selection, *Am Nat*, **104** (1970) 1-24.
2. Schaffer W M & Gadgil M, Selection for optimal life histories in plants, in *Ecology and evolution of communities*, edited by M Cody and J Diamond (Harvard University Press, Cambridge, Mass) 1975. 142-57.
3. Gadgil M & Prasad S N, Ecological determinants of life history evolution of two bamboo species of India, *Biotropica*, **16** (1984) 161-72.
4. Gadgil M, Male dimorphism as a consequence of sexual selection, *Am Nat*, **106** (1972) 574-80.
5. Gadgil M, Evolution of social behaviour through interpopulation selection, *Proc natn Acad Sci, USA*, **73** (1975) 1199-1201.
6. Gadgil S, Nanjundiah V & Gadgil M, On evolutionarily stable compositions of populations of interacting genotypes, *J theor Biol*, **84** (1980) 737-59.
7. Gadgil M, Joshi N V & Gadgil S, On moulding of population viscosity by natural selection, *J theor Biol*, **104** (1983) 21-42.
8. Gadgil M & Malhotra K C, Adaptive significance of the Indian caste system: An ecological perspective, *Ann Human Biol*, **10** (1983) 464-78.
9. Gadgil M & Malhotra K C, Ecology of a pastoral caste: the Gavli Dhangars of peninsular India, *Human Ecol*, **10** (1982) 107-43.
10. Malhotra K C, Khomne S B & Gadgil M, Hunting strategies among three non-pastoral nomadic groups of Maharashtra, *Man in India*, **63** (1983) 21-39.
11. Gadgil M & Prasad S N, *Conservation of bamboo resources of Karnataka*, Karnataka State Council for Science and Technology, Tech Report, 1981, pp 340.
12. Gadgil M, Prasad S N & Rauf Ali, Forest management in India: A critical review, *Soc Action*, **33** (1983) 127-55.
13. Gadgil M & Meher-Homji V M, Conserving India's ecological diversity, in *Conservation in developing countries*, edited by J C Daniel (Bombay Natural History Society, Bombay) in press.
14. Gadgil M & Malhotra K C, *A people's view of ecodevelopment*, Environmental Services Group, World Wildlife Fund, India, 1980, 1-26.
15. Prasad S N, Hegde M S, Gadgil M & Hegde K M, An experiment in ecodevelopment in Uttara Kannada district of Karnataka, *South Asian Anthropologist (Sp issue: Ecology is for the people)*, edited by K C Malhotra and M Gadgil, in press.



## B P Ghildyal

Rice ecosystem is the major source of protein and calories for 1,500 million low-income people of Asia. India ranks first in area under rice cultivation in the world and second in production; the yields per hectare, however, are among the lowest. Better understanding of this ecosystem is obviously vital for us.

Rice grows under diverse soil-climatic environments and often grows where no other crop will. In order to harvest good yields, we need to develop improved rice technology for such diverse environments.

Rice is primarily a tropical crop and scientific knowledge about tropical rice is much weaker than about other major cereals. In a tropical environment, hydrophysical and climatic constraints are difficult to change and are generally dominant. Lack or excess of water, high or low temperatures and soil constraints of alkalinity, salinity and acidity are widespread. Ghildyal and his coworkers have been directing their research efforts in this direction over the last three decades and have made significant contribution to our understanding of the physical environment of rice crop.

Rice plant and environment are coupled together in terms of energy and water flow. Necessary instrumentation was designed, fabricated and assembled locally for measuring environment radiation intensity, soil-plant-air temperature

profiles, humidity and wind profiles, plant characteristics like leaf area, size and orientation, leaf absorptivity, diffusion resistance to transpiration, root shoot conductivities and soil characteristics like oxygen diffusion rates, soil strength, soil thermal characteristics, soil-plant water potentials and water transfer in the whole soil-plant-atmosphere continuum.

Soil-plant-atmosphere system is extremely complicated and highly variable in space and time. For characterization of such a system, a large number of micro-meteorological, soil and plant parameters have to be measured and monitored continuously and integrated, processed and recorded simultaneously. For this purpose, a micro-processor based data acquisition system was designed and developed indigenously which could be operated under natural field situations.

Since plant characteristics like leaf shape and size are genotypical properties which affect the coupling with environmental parameters, a number of local, high yielding and stress tolerant genotypes were tested in hydrologically separated field lysimeters.

From these studies, critical limits of soil physical edaphic factors and micro-meteorological parameters affecting rice growth and yield have been identified. The mechanisms involved in the physical and chemical processes occurring in the

flooded and puddled soils and their relationship to rice growth and yield have been elucidated. As a result of these studies, several soil and water management practices have been suggested for increasing fertilizer and water use efficiency in rice culture. The results show that the age-old practices of puddling and flooding in rice culture are not *sine quo non* for successful rice production. Instead of puddling, soil compaction can meet the tillage requirements of flooded paddy. Soil compaction and flooding create a physical state of soil similar to that created by puddling and flooding of transplanted rice. In this system of cultivation, the soil is first ploughed and harrowed, fertilized and brought to an optimum soil moisture content for compaction (proctor moisture content). Heavy rollers or loaded tractor wheels are passed repeatedly over the soil surface till the desired bulk density can be achieved. The field can now be flooded and rice transplanted.

Basic studies on temporal and spatial variations in micro-climatological parameters and water-soil-plant relationship have thrown new light on the phenomenon of wilting in rice and the evapo-transpiration characteristics of rice crop, indicating that high water use efficiency plant types are available. The study also reveals the pathways of water in rice-soil system and methods to control water use without reducing the yields. We need now to begin to formulate and test complex models of rice plant communities, continuously checking theoretical models against field observations. We must continue to develop a strong theoretical and analytical basis of rice eco-system before we can advance significantly.

## Selected Publications

1. Ghildyal B P & Gupta U C, A study of the biochemical and microbiological changes during the decomposition of *Crotalaria juncea* (Sannhamp) at different stages of growth in soil, *Plant & Soil*, **11**(9) (1959) 312-30.
2. Ghildyal B P & Jana R K, Agro-meteorological environment affecting rice field, *Agron J*, **59** (1967) 286-87.
3. Varade S B & Ghildyal B P, Effect of bulk densities and seed placement on upland rice seedling emergence, *Agron J*, **60** (1968) 240-41.
4. Ghildyal B P & Mahajan K T, Investigation on the influence of oxygen levels and mechanical impedance as related to plant growth and tillage requirement for flooded paddy, *Annual Report on PL 480 Research Project A7-SWC-47*.
5. Chaudhary T N & Ghildyal B P, Influence of submerged soil temperature regime on growth, yield and nutrient composition of rice plants, *Agron J*, **62** (1970) 281-85.
6. Ghildyal B P, Soil and water management for increased water and fertilizer use efficiency for rice production, *Proc Int Symp Soil Fert Evaln*, New Delhi, 1971, 500-9.
7. Ghildyal B P, Soil-plant-atmosphere relationships in rice culture, *Annual International Rice Research Conference*, IRRI, Los Banos, Philippines, 1973.
8. Tomar V S & Ghildyal B P, Internal leaf water potential and transport of water in rice plants, *Agron J*, **65** (1973) 861-65.
9. Huda A K S, Ghildyal B P, Tomar V S & Jain R C, Contribution of climatic variables in predicting rice yield, *Agric Meteor*, **15** (1975) 71-86.
10. Kar S, Varade S B, Subramanyam T K & Ghildyal B P, Soil physical conditions affecting rice root growth, bulk density and submerged soil temperature regimes effects, *Agron J*, **68** (1976) 23-26.
11. Ghildyal B P, Field investigations of soil-water flux and evapotranspiration under different soil water regimes in the presence of shallow water table in mollisols of the subtropics, *Proc Conf on Role of physical properties in maintaining productivity of tropical soils*, International Institute of Tropical Agriculture, Ibadan, Nigeria, 6-10 December 1977.
12. Ghildyal B P, Effect of varying drainage conditions on water use and growth of rice, *Proc*



- Conf, International Institute of Tropical Agriculture, Ibadan, Nigeria, 6-10 December 1977.*
13. Ghildyal B P, Nature, physical properties and management of submerged rice soils, Vertisols and rice soils of the tropics symposia, *Proc 12th Int Cong Soil Sci*, New Delhi, 1982, 121-42.
  14. Ghildyal B P, Soil physical properties that affect rice root systems under drought, in: *Drought resistance in crops with emphasis on rice* (IRRI, Los Banos) 1982.
  15. Ghildyal B P, Physical characteristics of lowlands and traditional and new cropping patterns, *Proc International Conference on rainfed lowlands*, ICAR-IRRI, India, 1984.
  16. Ghildyal B P, *Effects of compaction and puddling on soil physical properties and rice growth* (Soils & Rice, IRRI, Los Banos, Philippines) 1978, 315-36.

## K S Gill

Gill passed his MSc (Agri) from the Punjab University in 1951 and got his PhD from California University in 1966. He has had a long and successful career as an agricultural scientist and administrator.

### Contributions as a Breeder

Wheat improvement work has been Gill's first love. He initiated composite crosses and recurrent selection for combining disease resistance and high yield potential in bread wheat. His work on durum wheat has been one of the many firsts that are to his credit. Again in triticale, it was for Gill to initiate the improvement work and lead it to a successful stage. Multiline as an approach in wheat improvement was put into practice for the first time under his leadership. Besides working for rust resistance through composite crosses and multilines, Gill's work on Karnal bunt disease is of outstanding importance. Due to this work, it is now becoming possible to develop varieties having genetic resistance against this disease. Intermating among the crosses involving lines with relatively less infection has been initiated in an effort to build up genetic resistance. As a result of the above-mentioned research efforts, a number of outstanding wheat and triticale varieties have been developed which are occupying vast acreage not only in the Punjab but in many other states also.

Some of the outstanding varieties developed by Gill are as follows:

*WL 711* was released in the Punjab in 1976 and in the north-western plains zone of India in 1979. It was also released in Pakistan. This is the highest yielding variety in the national programme for high-fertility irrigated conditions.

*WL 410* was released in the Punjab in 1977 and in the north-western plains zone of India in 1979 for cultivation under rainfed conditions. It combines resistance to rusts and loose smut diseases with high grain yield.

*KSML 3* was the first multiline variety to be released in the north-western plains zone of India in 1979; it has resistance to both yellow and brown rusts.

*DWL 5023* was the first semi-dwarf durum wheat variety to be released for irrigated high-fertility conditions in the northern plains zone of India in 1980. It has resistance to rusts, loose smut and Karnal bunt.

*PBW 34* is a durum variety identified for the north-western plains zone in 1982 and released for cultivation in the Punjab in 1983. With excellent grain quality, it possesses resistance to rusts, loose smut and Karnal bunt.

*WL 2265* was released in the Punjab in 1982 and was also recommended for the



north plains zone of India for cultivation under rainfed conditions.

WL 1562 (released in 1979), PBW 12 (released in 1982), Sonalika Multiline 1 (released in 1982), PBW 54 (released in 1983), PBW 120 (released in 1985) and PBW 138 (released in 1985) are the other varieties of wheat developed for cultivation in the Punjab.

TL 419 was the first triticale variety to be released for cultivation in the Punjab in 1981.

Gill has made significant contributions to bajra genetics and varietal improvement work. A number of new male sterile lines have been developed with three different sources of cytoplasmic-genetic male sterility. These new male sterile lines possess resistance to downy mildew which causes havoc to the hybrid bajra crop in the country. One of the new male sterile lines, Punjab 111-37A, has been utilized in the development of disease resistant hybrids. A resistant version of Tift 23A has been developed through recurrent selection. Rectification for resistance to downy mildew disease in male sterile lines, such as 110 and inbred lines BIL 38, has been achieved through mutagenesis. Another disease of bajra, ergot, was the main problem, particularly as no genetic sources of resistance were available. By using different screening techniques for inoculation with ergot spores and by selecting and intermating the materials with a low coefficient of infection, it has been possible to synthesize materials with better resistance. This is a new technique that has been adopted successfully to develop resistance to ergot in bajra. The research carried out on bajra has resulted in the development of a number of successful hybrids/varieties, such as PHB

10, PHB 14, PSB 8, PHB 47, etc. PHB 10 and PHB 14 are the first hybrids carrying genetic resistance to downy mildew and to be released at the national level. PSB 8 is the first composite variety released at the national level. PHB 47 is a hybrid with high yield and disease resistance. It has bristled ears which provide protection against bird damage.

Besides wheat and bajra, Gill has been responsible for the development of a number of varieties in other crops also (Barley-PL56; Sesamum-Punjab Til No. 1 and TL25; Linseed-LC45, LC54 and LC185; Guara Ageta Guara 111).

His work on cytogenetics and evolutionary relationship among various *Lincum* species is of outstanding merit and his book '*Linseed and Linseed Improvement*' (Indian Council of Agricultural Research) is a work of extreme scientific importance.

The contributions made by Gill have been recognized at the national and international levels by way of awards, honours and prizes conferred on him, including the Rafi Ahmed Kidwai Memorial Prize (1976); ICAR award for team research (1980); Group Award of Appreciation by the United States Department of Agriculture (1981); and Medal of the Mexican Government, CIMMYT, Mexico (1973). He was recognized as Professor of Eminence at national level by the Indian Council of Agricultural Research (1980). Gill has been the President of the Indian Society of Genetics and Plant Breeding, the Crop Improvement Society of India and Vice-President of the Genetic Association of India. He served as Session Chairman at the Sixth International Wheat Symposium held at Kyoto, Japan in 1983; International

Symposium on Potential Production of Field Crops under Different Environments held at IRRI, Manila, Philippines in 1981; and International Symposium on pearl millet held at ICRISAT, Hyderabad, India, in 1975.

Gill has published more than 300 research papers, over 100 popular articles and 12 books and bulletins.

### Contributions as a Teacher

Gill has contributed significantly to the undergraduate as well as postgraduate teaching of plant breeding and genetics. Besides giving a number of courses in these subjects, Gill has guided as many as 23 MSc and PhD students for their postgraduate research. He organized, as Director, four Summer Institutes sponsored by the Indian Council of Agricultural Research. Gill has also conducted four national level training programmes in wheat production technology sponsored by the United Nations Development Programme of the Food and Agriculture Organization and the Ministry of Agriculture, Government of India.

### Contributions as a Research Administrator

Gill has been actively involved in the administration of research. He was Head of Plant Breeding Department, Punjab Agricultural University for over 11 years. As many as 69 improved varieties of different crops were developed during this period. Besides creating new facilities for seed technology, tissue culture and quality testing, he strengthened the interdisciplinary and multilocational approach in plant breeding. The Plant Breeding Department has been acknowledged as an

outstanding department by many world recognized scientists who visited it.

During his tenure as Dean, College of Agriculture, Punjab Agricultural University (1979-83), the course curriculum of the college was revised and updated. Programme of work for starting a diploma course in agriculture was chalked out.

As Director of Research, Dr Gill is actively engaged in the overall guidance and direction of research projects in agriculture and allied sciences in the university.

### Selected Publications

1. Gill K S, *Research on dwarf wheats* (Indian Council of Agricultural Research, New Delhi) 1979, pp 180.
2. Gill K S, *Training manual for wheat production* (Ministry of Agriculture, Government of India, New Delhi) 1980, pp 308.
3. Gill K S, *Breeding methods for the improvement of pulse crops* (Indian Council of Agricultural Research, New Delhi) 1980, pp 433.
4. Gill K S, *Breeding oilseed crops* (Indian Council of Agricultural Research, New Delhi) 1980, pp 479.
5. Gill K S, *Linseed—A monograph* (Indian Council of Agricultural Research, New Delhi) 1981.
6. Gill K S, Bains K S & Chand K, Differential response of mutagens in inducing genetic variation in material traits in barley, *Z Pflanzen*, **71** (1974) 117-23.
7. Gill K S, Nanda G S, Gurdev Singh & Aujla S S, Studies on multilines of wheat (*Triticum aestivum* L). 1. Multilines and their components of Kalyansona cultivar, *Proc 3rd Intern Sabrao Congress*, Canberra, Australia, **4(a)**, 1977, 6-9.
8. Gill K S, Aujla S S, Sharma Y R, Nanda G S & Gurdev Singh, Studies on multilines in wheat (*T. aestivum*). 7. Seedling and adult plant reaction of components of Kalyansona and P V 18 multilines against yellow rust, *Cereal Res Commun*, **6(2)** (1978) 167-74.
9. Gill K S, Bhullar G S & Mahal G S, Gene action in a seven parent diallel cross of durum wheat (*Triticum durum* Desf), *Indian J agric Sci*, **49(11)** (1979) 827-31.



10. Gill K S, Nanda G S, Gurdev Singh & Aujla S S, Studies on multiline in wheat. 12. Breeding of a multiline variety by convergence of breeding lines, *Euphytica*, **29** (1980) 125-26.
11. Gill K S, Nanda G S, Gurdev Singh & Aujla S S, Multiline variety for controlling rust epidemics, *Ceres (FAO)*, **72** (1980) 1-3.
12. Gill K S, Grewal M S, Manmohan Singh & Sandha G S, Chromosome substitution in hexaploid triticale  $\times$  (Triticosecale Wittmack), *SABRAO J*, **13**(1) (1981) 33-38.
13. Gill K S, Nanda G S, Aujla S S, Singh G & Sharma Y R, Studies on multilines in wheat. 11. Progression of yellow rust in mixtures of resistant and susceptible components in varying ratios, *Indian J Genet*, **41** (1981) 124-29.
14. Gill K S, Nanda G S, Dhaliwal H S & Aujla S S, Studies to breed for resistance against Karnal bunt disease (*Neovossia indica*) in wheat, *Proc Sixth International Wheat Genetic Symposium*, Japan, 1983, 793-800.
15. Gill K S, Nanda G S & Gurdev Singh, Stability analysis over seasons and locations of multilines of wheat (*Triticum aestivum* L.), *Euphytica*, **22** (1984) 1-7.

## B M Gupta

The scenario during the closing stages of the second world war was one of great expectations of an early breakthrough and every success for chemotherapy of viral infections. The knowledge prevailing then in the field of bacteriology and contemporary virology made it look so. The antibiotics had made their appearance to show that these drugs readily combined with and inactivated bacteria directly in preference to the host invaded and it was considered only a matter of time for similar antibiotics to be discovered against viruses as well. Also, powerful and chemically diverse types of plant virus inhibitors, namely, trypsin, ribonuclease and phytolectin glycoprotein, studied at the time, were all having the property to combine readily with virus particles *in vitro*. To this could be added the available evidence from serology that specific virus antisera would need to combine with virus in fixed ratios for neutralization of virus infectivity *in vivo*. From this, a consensus had developed in the early mid-forties that the ability of an antiviral substance (or a virus inhibitor) to combine with virus particle was probably a prerequisite for the inhibition of virus infectivity expressed *in vivo*. This hypothesis, however, became suspect as all hopes of finding nontoxic antiviral antibiotic(s) receded fast with the results of screening coming in one after the other from different laboratories of the

world, each drawing a blank. Indeed, Dr Thomas Rivers of the Rockefeller Institute of Medical Research is on record having deprecated as early as in 1948 the indiscriminate use of antibiotics in viral infections [Rivers T, *Viral and Rickettsial Infections of Man* (G B Lippincott, P A) 1948, 15].

### Discovery of Antiviral Agents (Substances Acting via Host)

In a renewed bid to detect antiviral substances from fungi, the growth products of 49 species<sup>1,2</sup> of fungi were tested for their effect on the infectivity of tobacco mosaic virus (TMV) in plants used as a model, and also against a number of related and unrelated plant viruses. A complex polysaccharide, freed of a dialysable phytotoxic component\* present in mixture with it, was isolated from the culture filtrate of mould fungus *Trichothecium roseum* and was shown to be capable in a dose dependent manner of inhibiting a broad range of related and

---

\*Chemically identified by Bawden and Freeman to be trichothecin (C<sub>19</sub>H<sub>24</sub>O<sub>5</sub>), a sesquiterpenoid [*J gen Microbiol*, **7** (1952) 154-68] and a major component of the "yellow rain" which allegedly was used as a chemical warfare agent in Asia [See citation in Chessin M, *Bot Rev*, **49**(1) (1983) 16; and *Microbial toxins*, Vol 7, *Fungal Toxins*, edited by S Kadis *et al.* (Academic Press, New York) 1971, 253].



unrelated plant viruses rather *indirectly* via action directed principally against the host. The resistance acquired at the site of treatment of leaves<sup>2</sup> (lower surface) travelled away from it to a remote site (upper surface).

The complex polysaccharide, a heat stable substance containing traces of double-stranded RNA<sup>3</sup>, later given by us the trivial name T-poly<sup>3-5</sup>, was shown to be nontoxic for the host and a potent inhibitor of virus infection. It possessed no ability to combine with or inactivate viruses *in vitro*. Treated hosts became insusceptible to infection either completely or partially, depending on the specific identity of the test plant, the dose of T-poly and the length of time it remained in contact with the host. Quantitative inhibitor-virus interaction studies conducted in accordance with the laws of mass action on one hand and the demonstration of restoration of infectivity on the other by the process of simple dilution or ultracentrifugation<sup>6,7</sup> of the noninfective virus and T-poly admixture also supported the hypothesis and the inescapable conclusion that the antiviral effect of T-poly is exerted indirectly via the host. The fungus *T. roseum* provided the source for the first report and demonstration of the existence in nature of antiviral substances acting indirectly via the host to produce the effect. Antiviral substances acting via the host were given the class name 'antiviral agents'<sup>1,6</sup>. The discovery of antiviral agents with the possibilities of prohost approach to control and management of viral infection, long before the true nature of virus multiplication was deciphered, went unheralded at the time, even though it really marked a departure from the critical concept prevailing then from the days of

penicillin that antiviral substances to be effective must combine with and directly inactivate viruses in preference to the host. While reviewing this contribution to knowledge on virus inhibitors, Sir Fredrick C Bawden, writing in *Advances in Virus Research* (Vol II, Acad. Press, 1954, 50-52), is on record having commented that it was unfortunate that all virus inhibitors chosen for work earlier had opposite electrical charges around pH neutrality at which they combined with viruses and it was impossible to say then that the combination did not play any part in the overall process of virus inhibition.

### Vertebrate System

Adequacy of the prohost approach to virus chemotherapy was amply substantiated soon thereafter with the discovery in different laboratories of the world that fungi yielded antiviral substances (e.g. statolon and helenin) that inhibited vertebrate viruses also indirectly via the host in a dose dependent manner [Powell H M *et al.*, *Antibiot & Chemoth*, **2** (1952) 432]. The prospects for prohost antiviral chemotherapy brightened further with the discovery in 1957 of the antiviral agent interferon and the finding that the antiviral effect of interferon is also exerted indirectly via the host [Taylor J, *Biochem biophys Res Commun*, **14** (1964) 447]. Subsequent knowledge that a variety of interferon types (type 1 and type 2) and at least two dozens subtypes [Merigan T C, *New Engl J Med*, **308** (1983) 1530] can be induced in vertebrate cells by viral and nonviral stimuli [Johnson H N & Baron S, *Adv exp Med Biol*, **10** (1978) 25] and that besides being antiviral interferons and interferon inducers possess additionally immunoregulatory and inducing ability for a variety of host biological functions, had

served only to widen the scope of prohost approach to antiviral chemotherapy. Two major constraints, namely, hyporesponsiveness to repeated administrations and low margin of safety, thwarted development and use of interferon inducers (e.g. poly I:C) as suitable antiviral or anticancer agents in practical medicine.

#### **6-MFA: A New Interferon Inducing Antiviral Agent with Immunoregulatory and Anticell Proliferative Potentials from *Aspergillus ochraceus* ATCC 28706**

6-MFA, chemically a complex polysaccharide in mixture with a mycovirus or virus-like particles (VLP), having multisegmented double stranded RNA genomes, was extracted by us at the Central Drug Research Institute (CDRI) from the mould fungus *Aspergillus ochraceus* ATCC 28706 (Syn. *A. flavus*, DU/KR/162b). 6-MFA treated animals (or cells in culture) resist a variety of related and unrelated human and animal virus challenge infections that include arboviruses (Japanese encephalitis, Kysanur forest disease virus parainfluenza-3 herpes 1, foot and mouth, measles and coxsackie-4<sup>8-11</sup>).

Physically, the multisegmented dsRNA's<sup>12,13</sup> of varying molecular weights are coated with polypeptides to form the VLP and many such VLP's (6% w/w) constituting the mass of 6-MFA in mixture with the polysaccharide (80-90% w/w). The dsRNA (15 mg 6-MFA = 100  $\mu$ g dsRNA) is the essential component responsible for the antiviral resistance-inducing activity of 6-MFA and the polypeptide contributes to the stability of dsRNA. The polysaccharide component is linked with the immunoregulatory activity in 6-MFA; the

precise mechanism of the association is unclear at the moment.

According to Prof. de Clercq of Leuven University, Belgium, who did preliminary tests with 6-MFA sample sent from CDRI in tissue culture model, 6-MFA is not cytotoxic. The antiviral spectrum of 6-MFA extends beyond arboviruses to include unrelated enteroviruses and respiratory viruses as well (Personal communication, 7 May 1982).

Tests conducted at CDRI by us and at the National Institute of Virology, Pune, showed that 6-MFA induces in test mice the production of two types of interferons, pH 2 stable and pH 2 labile, suggesting that this antiviral agent may be a broad spectrum interferon inducer<sup>14-18</sup>. That most of the acid labile interferon induced may be of immune (gamma) variety is suggested from the tests of neutralization of antiviral activity carried out with mixtures of  $\alpha$ -,  $\beta$ - and  $\gamma$ -interferon antisera<sup>19</sup>. Combination of  $\alpha$ - and  $\beta$ -antisera alone, under the conditions permitting complete neutralization of the activity of poly I:C, would account for only a part of the abrogation of the activity of 6-MFA. Both the quality and quantity of interferons induced by 6-MFA can be altered sequentially by administering orally a glycosidic extract of the medicinal plant *Panax ginseng*, resulting in considerable enhancement of antiviral activity<sup>14,15</sup>, indicating that 6-MFA can act additively or synergistically in concert with the pharmacologically active substances from plants.

The mouse protective activity of 6-MFA against Semliki forest virus was found by us to depend on the concomitant expression of immune response of the



host acting in concert with the interferon(s) induced. This became evident from the response of the host mice given the combined treatment of 6-MFA with an immunosuppressor (cyclophosphamide), which caused abolition of the antiviral resistance inducing activity of the inducer<sup>13</sup>. This aspect of the antiviral potentialities of 6-MFA was investigated in greater depth by groups of workers from USA, School of Medicine and Dentistry, Georgetown University, Washington D C, Uniformed Services University of Health Sciences, Bethesda, Maryland, and National Center of Drugs and Biologics, Maryland, USA. These workers not only confirmed the earlier results published by us from CDRI, but also showed that tunicamycin (TM), an antibiotic widely accepted as one that inhibits glycosylation and membrane-associated glycoproteins relevant in almost all respects of the immune system, reverses the antiviral (SFV) resistance inducing activity of 6-MFA in intact host (mice). [See Maheshwari *et al.*, *Infect Immun*, **41**(1) (1983) 61]. The exact mechanism of immunological modulation as the basis of reversal by tunicamycin of 6-MFA induced mouse protection is unclear at this point of time.

A fuller understanding of the mechanism of the reversal of mouse protection by tunicamycin and knowledge of the types and subtypes of interferon selected out by tunicamycin treatment in 6-MFA treated mice may provide a solution to the problem of hyporesponsiveness to repeated administrations of 6-MFA in virus (SFV) infected mice<sup>20</sup>. Three recent findings from our laboratory provide certain clues in this direction: (i) Thymus dependent sheep red blood cell (SRBC) antibody and the plaque forming cell

response to SRBC antigen in test animals (mice) are both stimulated or inhibited depending on the time of administration of 6-MFA in relation to the antigen<sup>15,16,18</sup>, (ii) cell-free thymus extract prepared from sensitised mice (survivors of SFV) would help to restore the depression of protection rate initiated by tunicamycin, and (iii) tests at Bhabha Atomic Research Centre, Bombay, have revealed that 6-MFA (sample sent from CDRI) has a marked antitumor (lymphosarcoma) effect in murine model (Poduval T B, Personal communication, 4 December 1984).

Immunomodulatory and anticell proliferative potentials of 6-MFA, both being host directed, would be expected to influence host functions in vertebrates that may or may not be concerned with defence against viruses or cancer alone. Indeed, a recent study carried out at the Industrial Toxicology Research Centre, Lucknow, has shown that 6-MFA treatment can help reduce in rats to a significant extent the cytotoxic effect of benzene (a known immunosuppressive and leukemogenic agent), a function that is associated with amelioration of lipid peroxidation and iron accumulation (occurring as a result of benzene toxicity) and increase in superoxide dismutase activity [*Proc Soc Biol Chem Meeting*, Delhi, 12-14 October 1984, Abstr 625]; *Toxicology*, **39** (1986) in press].

## Summary

Viruses can be regarded as dangerous not for what they are ("vagabond genes") but for what they do to us once inside our system. Current portents compel us to engage and fight viruses on all fronts, from viruses that prefer to localise themselves in particular organs or tissues (brain, liver,

lung and skin), to those that challenge and cause the entire cellular host immune system to collapse (acquired immune deficiency syndrome, AIDS). Bioproducts or chemicals with capacity to induce in our body production of different types of interferons (*acid stable* and *acid labile*) and in right proportions, and also having the power to regulate the host's immune response, are far more likely to succeed in clinical practice as an effective antiviral agent than a whole coterie of directly acting virucidal substances. 6-MFA has been found to possess many of the attributes of an effective antiviral agent, combining with itself the anticell proliferative properties<sup>21</sup> and the ability as well, either by itself<sup>21</sup> or in association with diverse range of immunostimulators<sup>14,22,18</sup>, to alleviate certain forms of immunosuppression.

### Selected Publications

1. Gupta B M, Studies on antiviral agents from fungi, *Master's degree thesis, University of Pittsburgh, USA*, 1948.
2. Gupta B M & Price W C, Production of plant virus inhibitors by fungi, *Phytopathology*, **40** (1950) 642-52.
3. George C X, Gupta B M, Khurana S M P & Nagaic B B, Antiviral activity in plants of mycoviral double-stranded RNA from *Trichothecium roseum*, *Acta Virol*, **25** (1981) 408-14.
4. Gupta B M, Chandra K, Verma H N & Verma G S, Induction of antiviral resistance in *Nicotiana glutinosa* plants by treatment with *Trichothecium* polysaccharide and its reversal by Actinomycin D, *J gen Virol*, **24** (1974) 211-13.
5. Gupta B M, Inhibition of plant virus infection by antiviral agents, in *Aphids as virus vector*, edited by Kerry Harris and Karl Maramorosch (Academic Press, New York) 1977, Chap 19, 455-71.
6. Gupta B M, Mechanism of inhibition of plant virus infection by fungal growth products, *Pittsburgh Univ Bull*, **47**(5) (10 April 1951) 2.
7. Gupta B M & Price W C, Mechanism of inhibition of plant virus infection by fungal growth products, *Phytopathology*, **42** (1952) 45-51.
8. Maheshwari R K & Gupta B M, Antiviral agents from fungi effective against Semliki forest virus (SFV) in mice, *Indian J med Res*, **61** (1973) 1292-96.
9. Maheshwari R K, Hussain M M & Gupta B M, 6 MFA, an antiviral agent from *Aspergillus ochraceus* ATCC 28706: Influence of body weight and mineral oil administration on the antiviral activity in mice, *Acta Virol*, **21** (1977) 63-70.
10. Maheshwari R K, Gupta B M, Ghosh S N & Gupta N P, Antiviral agent (6-MFA) from *Aspergillus ochraceus*—Sensitivity of arboviruses in experimentally infected mice, *Indian J med Res*, **67** (1978) 183-89.
11. Gupta B M, Interferon inducing antiviral agents from fungi, *Indian J Microbiol*, **18** (1978) 203-20.
12. Kong T, Bozarth R F & Gupta B M, Properties of virus like particles in *Aspergillus ochraceus*, Abstracts, American Society for Microbiology, Spring Meeting, Indiana, 14 April 1979.
13. George C X & Gupta B M, Mycoviral ds-RNA, an interferon inducing antiviral agent cannot protect cyclophosphamide treated mice against challenge from Semliki forest virus (SFV) infection, *Interferon Scientific Memorandum*, March 1981.
14. Singh V K, George C X, Singh N, Agarwal S S & Gupta B M, Combined treatment of mice with *Panax ginseng* extract and interferon inducer, *Planta Medica*, **47** (1983) 234-36.
15. Singh V K, George C X, Singh L M & Gupta B M, Immunomodulatory activity of 6-MFA, an interferon inducing antiviral substance from *Aspergillus ochraceus* ATCC 28706, *Indian J Parasit*, **7** (1983) 225-29.
16. Dusre Lata, Singh V K & Gupta B M, Haemagglutinating antibody and plaque forming cell response to sheep red blood cell antigen in mice treated with 6-MFA and thymic extract, *Indian J Parasit*, **8** (1984) 145-49.
17. Ghosh S N, Goverdhan M K, Cecilia D, Chelliah S, Kedarnath N & Gupta B M, Protective effect of a fungal interferon inducer (6-MFA) against Japanese encephalitis infection in mice, *Indian J med Res*, **79** (1984) 705-8.
18. Singh V K, Agarwal S S & Gupta B M, Immunomodulatory activity of *Panax ginseng* extract, *Planta Medica*, **50** (1984) 462-65.



19. Gupta B M & Singh V K, Interferon inducing antiviral agent from *Aspergillus ochraceus* ATCC 28706, Abstract No 105999, *Sixth International Congress of Virology*, Sendai (Japan), 2 September 1984.
20. Gupta B M, *Developments in antiviral chemotherapeutic research*, 22nd Prof. B B Mundkur Memorial Lecture, Indian Phytopathological Society, Lucknow, 9 January 1985.
21. Pandya K P, Shankar R, Gupta A, Khan W A & Ray P K, Modulation of benzene toxicity by an interferon inducer (6-MFA), *Toxicology*, **39** (1986) in press.
22. Dusre L, Gupta B M, Singh V K & Maheshwari R K, Mouse protection against Semliki forest encephalitis virus by 6-MFA; Abrogation by tunicamycin and its reversal by thymic extract, *Indian J Parasitol*, **9**(2) (1985) 193-98.

---

Profiles in Scientific Research : Contributions of the Fellows, Vol. 2 (Indian National Science Academy, New Delhi), 1986.

## H K Jain

Jain started his research career in the mid 1960's as a cytologist and one of his earliest interests was in studying the role of the nucleolus synthesizing locus. It was found that this cytologically identifiable locus has a profound effect on chromosomal development during meiosis. Using trisomic lines and tritium autoradiography, the locus was shown to be hyperactive in RNA synthesis. Later studies by molecular biologists were to show that the nucleolus organizer of the cytologist is made of amplified cistrons responsible for the production of ribosomal RNA. Using the tritium label, Jain and his collaborators were the first to identify four such loci in the chromosomes of bread wheat—a polyploid with three genomes, A, B and D. This was done using ditelosomic lines developed by Sears. The four loci in the different genomes showed differential activity, suggesting interaction. A related study on RNA synthesis showed that the antimetabolite actinomycin D would transform meiotic cells into mitotic in the desert locust, apparently by blocking the synthesis of key enzymes.

The second group of studies started in the 1960's were concerned with the cytological basis of genetic recombination and regulation of the extent of recombination at the interchromosomal level in a genome. During this period, there was a great deal of skepticism about the classical hypothesis of genetic

recombination, which suggested breakage and reunion of chromatids. Using *Delphinium* with its differentiated chromosomes as a test organism, Jain and his students showed that the classical theory is basically true. Further analysis demonstrated that each chromosome pair in a genome shows some extent of recombination independently of other pairs (the autonomous fraction), and that there is a second fraction (the correlated fraction), for which the different pairs appeared to "compete". This was a cytological manifestation of the control of the extent of genetic recombination in a genome. A series of papers were published on this theory in *Chromosoma*, *Heredity* and *Nature*.

Later in the 1960's, Jain's group at the Indian Agricultural Research Institute became interested in mutagenesis, but from an applied point of view very little came out of this work. There were, however, two interesting observations. First, LSD was shown to be effective in inducing chromosomal aberrations in barley. There had been some controversy regarding this, but their observations published in "Science" set these doubts at rest. The second observation related to mutagen specificity, which is well recognized now. His group was one of the first to show that base specific chemicals like hydrazine and hydroxylamine give a different spectrum of mutations for



different loci. A number of marker genes were selected for this purpose in tomato, and it was shown that some of the loci (e.g. *d* giving dwarf mutations) were more susceptible to hydrazine than hydroxylamine. Similar results were obtained in *Drosophila*. The experiments on *Drosophila* were repeated in Prof. Aurbach's laboratory in Edinburgh, using a better technique and these confirmed the main finding.

Some of this work on induced mutagenesis carried out by Jain's graduate students has helped to develop selection procedures for induced variations affecting quantitative traits of economic value.

In more recent years, Jain has been interested in applied genetics, more particularly in the theory of selection pressures for crop yields. In a series of papers, he and his collaborators have argued that crop plants in traditional agriculture have been selected primarily for high dry matter production associated with an aggressive growth habit. This helps them to survive in stress environments. The basic approach for higher crop yields in recent years has been to reconstruct these plants genetically so as to select for a high harvest index. While this remains a valuable tool, Jain's group has argued that there are limits to improvements through this means and that the emphasis once again will have to shift to increased dry matter production through the manipulation of photosynthetic activity, if the emerging yield ceilings are to be overcome. A related theme which has received a great deal of attention concerns the problem of increasing genetic uniformity of crop plants and the threat of disease and pest epidemics. Jain has proposed the concept of a multilineal

complex of genetically diverse varieties distributed in time and space to create genetic barriers against the build-up of pests and pathogens. Another aspect of applied work carried out by his team at the Indian Agricultural Research Institute relates to breeding for higher protein content in cereals. A new selection procedure based on experimental studies on wheat has been proposed. The new procedure has resulted in the development of a high protein genetic stock of wheat of good breeding value.

Jain's future work would be in the area of policy, planning and strategy for research in the context of India's growing food needs. Most of the present gains in agricultural production in India have come from favourable genotype-environment interactions. These interactions work best in fertile soils provided with irrigation and other inputs. However, the country must increasingly think of more than 50% of its lands where these interactions could not be generated because of moisture stress and other limitations. Research planning for these situations and for renewable resources of energy in agriculture should emerge as an important priority for him and his colleagues.

### Selected Publications

1. Jain H K, India's coordinated crop improvement projects—organization and impact, *Indian Fmg*, **34**(4) (1984) 3-9, 27-37.
2. Jain H K, Scientific transformation of Indian agriculture—The second phase, *Curr Sci*, **52** (1983) 528-34.
3. Jain H K, Future agricultural technology : India looks ahead, *Interdisciplinary Sci Rev, Lond*, **7**(3) (1982) 164-66.
4. Kulshreshtha V P & Jain H K, Eighty years of wheat breeding in India : Past selection pressures and future prospects, *Z Pflanzen, Berl*, **89** (1982) 19-30.

5. Jain H K, Genetic reconstruction of pulses for increased production and intensive cropping, *Indian Fmg*, **31**(5) (1981) 7-11; 86.
6. Singhal N C & Jain H K, Evolution of a high protein genetic stock of bread wheat, *Z Pflanz, Berl*, **87** (1981) 186-92.
7. Jain H K, Scientific research and technological developments in developing countries : Achievements and failures in agriculture, *Soc & Sci*, **3**(4) (1980) 81-90.
8. Jain H K, Incidental DNA, *Nature, Lond*, **288**(5792) (1980) 647-48.
9. Jain H K & Shankar K, Strategy for improvement of grasslands and fodder resources of India, *Indian J Genet*, **37**(2) (1977) 159-71.
10. Jain H K, Singhal N C & Austin A, Breeding for high protein yields in bread wheat : Experimental approach and a phenotypic marker, *Z Pflanz*, **77** (1976) 100-11.
11. Jain H K & Kulshreshtha V P, Dwarfing genes and breeding for yield in bread wheat, *Z Pflanz*, **76** (1976) 90-101.
12. Jain H K, Mukherjee B K, Singh R D & Agarwal K N, The present basis and future possibilities of breeding for yield in maize, *Z Pflanz*, **76** (1976) 102-12.
13. Jain H K & Raut R N, Regulation of RNA synthesis in the evolution of bread wheat, in *Chromosomes Today*, Vol 3, edited by C D Darlington and K R Lewis (Oliver and Boyd, Edinburgh) 1972, 53-55.
14. Jain H K & Shukla P T, Locus specificity of mutagens in *Drosophila*, *Mutat Res*, **14** (1972) 440-42.
15. Singh M P, Kalia C S & Jain H K, Chromosomal aberrations induced in barley by LSD, *Chromosoma*, **32** (1970) 142-51.
16. Jain H K & Singh U, RNA synthesis and chromosome behaviour in *Scistocerca*, in *Chromosomes Today*, Vol 2, edited by C D Darlington and K R Lewis (Oliver and Boyd, Edinburgh) 1969, 70-74.
17. Jain H K, Raut R N & Nerwal S K, Nucleolar organizer as a hyperactive locus for RNA synthesis, *Heredity*, **24** (1969) 59-67.
18. Jain H K, Singh M P & Raut R N, Genome differentiation and RNA synthesis in wheat, *Proc 3rd Int Wheat Genet Symp*, Canberra, 1968, 379-84.
19. Jain H K, Raut R N & Khamankar Y G, Base specific chemicals and mutation analysis in *Lycopersicon*, *Heredity*, **23** (1968) 247-56.
20. Jain H K & Raut R N, Differential response of some tomato genes to base specific mutagens, *Nature, Lond*, **211** (1966) 652-53.
21. Basak S L & Jain H K, The interchromosome distribution of chiasmata in interchange heterozygotes of *Delphinium*, *Heredity*, **19** (1964) 53-61.
22. Jain H K & Basak S L, Genetic interpretation of chiasmata in *Delphinium*, *Genetics*, **48** (1963) 329-39.
23. Jain H K & Basak S L, Interchromosome effects of chiasmata and crossingover, *Nature, Lond*, **197** (1963) 725-26.
24. Basak S L & Jain H K, Autonomous and interrelated formation of chiasmata in *Delphinium* chromosomes, *Chromosoma*, **13** (1963) 577-87.
25. Jain H K & Maherchandani N, The control of intranuclear distribution of chiasmata in *Delphinium*, *Heredity*, **16** (1961) 383-92.
26. Jain H K & Bose A K, Experimentally induced interbivalent redistribution of chiasmata in *Delphinium*, *Nature, Lond*, **186** (1960) 260-61.
27. Jain H K, Correlated failure of synthetic activities in a wheat chromosome, *Nature, Lond*, **182** (1958) 1458-59.



## J S Kanwar

The main research contributions of Kanwar have been in the following subject areas: (1) Soil fertility and fertilizer management; (2) Micronutrients; (3) Sulphur; (4) Water management; (5) Soil salinity and alkalinity; (6) Dryland agriculture; and (7) Environment.

### Soil Fertility and Fertilizer Management

Improving soil fertility and scientific management of fertilizers and efficiency of fertilizer use for increasing crop production had been the main target of Kanwar's researches in Punjab, where he spent the first 20 years of his scientific career. He established the need for P and K besides N for obtaining satisfactory yields of crops under both irrigated and unirrigated conditions. He emphasized the need for balanced use of fertilizers keeping in view the soil and crop needs. He published more than 70 papers on this subject, besides the book '*Soil fertility—Theory and practice*', which provides a sound Indian experience on modern agriculture.

### Micronutrient Research

Kanwar and N S Randhawa's pioneering research on micronutrients opened up a new chapter in Indian agriculture and has led to the use of micronutrients under intensive agriculture. The monitoring of

micronutrient status has shown that micronutrient deficiencies are increasing in intensity and extent under exploitive agriculture. He has built a strong school of micronutrient research in India. His papers<sup>7-12</sup> indicate the leads in micronutrient research developed by him.

### Research on Sulphur in Soil and Crop Nutrition

Kanwar recognized sulphur deficiency in the groundnut and tea growing areas of Punjab and demonstrated the effect of S-containing fertilizers in increasing yields of crops. His researches built up a team of scientists in Punjab who have followed the research leads developed by him. Kanwar has predicted that intensive cropping, use of high analysis and low S-containing fertilizers and removal of crop residues, are creating S deficiency, which can be met only through the use of S-containing substances, such as gypsum, phosphogypsum, pyrite and other sources of sulphur. He has drawn attention of the fertilizer industry to the necessity of increasing S content of fertilizers for correcting S deficiency, which is affecting the production of crops seriously, specially under a system of intensive cropping<sup>13-15</sup>.

Kanwar and M S Mudahar have critically analyzed the S problems of the tropical countries<sup>16</sup>.

---

Dy-Director General (Research, Coordination and Training), International Crops Research Institute for the Semi-Arid Tropics, Hyderabad-502324; Residence : Hasan Vila, 8-2-595/2, Banjara Hills, Road 10, Hyderabad-500034.

## Water Management

Over the years, Kanwar has developed the philosophy that irrigation water alone is not enough and the cause of low payoff from costly irrigation projects in India is the neglect of scientific water management and the resultant poor drainage, soil salinity and alkalinity<sup>17-20</sup>.

## Soil Salinity and Alkalinity

Kanwar's work on saline alkali soils in Punjab led to the development of a new technology for the reclamation of such deteriorated soils which cover many million hectares in this country<sup>21-23</sup>.

He has been a crusader for the cause of soil science research in India. In his presidential address at the Indian Society of Soil Science in 1971, he focussed attention on problems of the future<sup>27</sup>.

## Dryland Agriculture

The future of the world, particularly of India, lies in increasing the productivity of dryland agriculture, which covers more than three-fourths of the cultivated areas in India. Kanwar focussed attention on dryland agriculture research and after organizing a coordinated programme of research on dryland agriculture in India under the ICAR, he became the first Director of Research at ICRISAT, which has main focus of research on dryland agriculture for the semi-arid tropics<sup>27-29</sup>.

## Research on Environment

Kanwar, in his presidential address to the 12th International Congress of Soil Science in 1982, focussed attention on managing soil resources to meet the challenges to mankind. Several of his papers<sup>35-41</sup> cover various aspects and problems of environment.

The following concluding words of his address to the 12th International Congress of Soil Science sum up his ideas about the role of soil science research and education in the world of today and of tomorrow :

"The soil scientists must recognize soil and society as a single system in which man can be either a positive or a negative factor, depending upon how he acts. Today's world is conscious of the energy crisis and has a slogan "Save the oil", but we seldom hear the slogan "Save the soil".

With today's technology, we can certainly modify soils to man's advantage. We can predict the changes and improvements if we know our soil well. We could computerize irrigation schedules, fertilizer needs and the likely response of soils to management. I consider the 1980s a decade of integrated soil management systems or better soil-water-plant management systems in which all scientists, planners and farmers work together to save the soil, improve its health and make it produce to its potential capacity, which is very high.

Whether there are disasters like floods or droughts, food or water famine, energy crises or health hazards, poor soil management is the cause. We have the capacity to improve the situation, but we need the will to do it and a national and world soils policy to accomplish it."

Kanwar has published around 275 scientific papers and has contributed to several books, individually or jointly with his colleague scientists. The awards won by him include : (i) Rafi Ahmed Kidwai Memorial Award for research in soil science (1967); (ii) Borlaug Award for research in agriculture (1977); (iii) Dhirumal Murarji Award for fertilizer



research (1982); (iv) International Fertilizer Development Centre Century Club Award for research on sulphur (1983); and (v) Indian Society of Soil Science Award for distinguished service to soil science (1984).

### Selected Publications

1. Kanwar J S, Phosphate retention in some Australian soils, *Soil Sci*, **82**(1) (1956) 43-50.
2. Kanwar J S & Grewal J S, Phosphate fixation in Punjab soils, *J Indian Soc Soil Sci*, **8** (1960) 211-18.
3. Kanwar J S & Prihar S S, Effect of continuous application of FYM and inorganic fertilizers on crop yields and properties of soil. I. Chemical properties, *J Indian Soc Soil Sci*, **10** (1962) 109-20; II. Physical properties, *J Indian Soc Soil Sci*, **10** (1962) 243-47.
4. Kanwar J S & Grewal J S, Direct and residual availability of native and applied potassium in Soil, *J Res PAU*, **3**(1) (1966) 1-6.
5. Kanwar J S, High yielding varieties: Twin essentials for maximum yield-use of balanced fertilizers, scientific methods for soil and water management, *Indian Fmg*, **18**(3) (1968) 4-5.
6. Kanwar J S, Balanced fertilizer use—The key to agricultural production, *Indian Fmg*, **20**(11) (1971) 7-9.
7. Kanwar J S, Influence of organic matter on copper fixation of the soil, *J Indian Soc Soil Sci*, **2** (1954) 73-80.
8. Kanwar J S, Direct and residual effect of major and micronutrients applied to berseem in a sandy loam soil, *Indian J Agron*, **2**(2) (1962) 112-18.
9. Kanwar J S, Research on trace elements in Punjab—Present and future, *J Indian Soc Soil Sci*, **12** (1964) 221-24.
10. Kanwar J S & Randhawa N S, Micronutrient research in soils and plants in India—A review, *ICAR tech Bull (Agric)* No 50, 1967.
11. Kanwar J S & Mehta K K, Toxicity of fluorine in some well waters of Haryana and Punjab, *Indian J agric Sci*, **38**(5) (1968) 881-86.
12. Kanwar J S & Youngdahl L J, Micronutrient needs of tropical food crops, *Fertil Res*, **7**(1) (1985) 43-67.
13. Kanwar J S, Investigation on sulphur in soils. I. Sulphur deficiency in groundnut soils of Samrala (Ludhiana), *Indian J agric Sci*, **33**(3) (1963) 196-98.
14. Kanwar J S & Takkar P N, Responses to sulphur in tea soils of the Punjab, *J Res PAU*, **3**(3) (1966) 246-52.
15. Kanwar J S, Sulphur deficiency—A key factor in Indian agriculture, *Fertil News*, **12**(6) (1967) 9-12.
16. Kanwar J S & Mudahar M S, Fertilizer sulfur and food production—Research and policy implications for tropical countries, *IFDC Bull* No 28, 1984.
17. Kanwar J S, From protective to productive irrigation, *Econ Polit Wly*, **4**(13) (1969) 22-26.
18. Kanwar J S, New dimensions in water management technology for agriculture in India, *Symposium on Soil and Water Management*, ICAR Bull, 1972.
19. Kanwar J S, Soil and water—Looking ahead: Presidential address, *J Indian Soc Soil Sci*, **20** (1972) 199-206.
20. Kanwar J S, Water management and crop planning in India, *Proc Regional Workshop on Irrigation Water Management* (Asian Development Bank) 1973, 226-41.
21. Kanwar J S, Quality of irrigation water as an index of its suitability for irrigation purposes, *Potash Rev*, **24** (1960) 13th Suite, 1961.
22. Kanwar J S & Shah Singh S, Boron in normal and saline-alkali soils of the irrigated areas of the Punjab, *Soil Sci*, **92**(3) (1961) 207-11.
23. Kanwar J S, Clay minerals in saline-alkali soils of the Punjab, *J Indian Soc Soil Sci*, **9** (1961) 35-40.
24. Kanwar J S, Reclaiming deteriorated soils, *Fertil News*, **6**(2) (1961) 19-21.
25. Kanwar J S, Reclamation of saline alkali soils—Amendments, kinds, amounts and costs, *Proc Seminar on Saline and Alkali Soils Problems*, New Delhi, 1962, 61-67.
26. Singh N T, Bhumbra D R & Kanwar J S, Effect of gypsum alone and in combination with plant nutrients on crop yields and amelioration of a saline-sodic soil, *Indian J agric Sci*, **39**(1) (1969) 1-9.
27. Kanwar J S, Challenge to soil scientists in the seventies, *J Indian Soc Soil Sci*, **19**(3) (1971) 221-26.
28. Kanwar J S & Bhumbra D R, Manuring of wheat under unirrigated conditions in the Punjab, *Indian J Agron*, **4** (1959) 74-81.
29. Kanwar J S, Agricultural development in India, in *Arid Lands in Transition*, edited by Harold E Dregne, Publ No 90 of the American Association for the Advancement of Science, Washington, DC, 1970, 451-70.

30. Kanwar J S, Dryland agriculture and fertilizer use—Present and future prospects, *Proc FAI National Seminar on Fertilizers in India in the Seventies*, 18-19 December 1972, 11-2/1-17.
31. Kanwar J S, The Third R V Tamhane Memorial Lecture—Soil and water management—The key to production in rainfed agriculture of semi-arid tropics, *J Indian Soc Soil Sci*, **24**(2) (1976) 230-39.
32. Kanwar J S, Rainwater and dryland agriculture—An overview, *Symposium on Rainwater and Dryland Agriculture*, New Delhi, October 1980, Indian National Science Academy, New Delhi, 1980.
33. Kanwar J S, Dimensions of problems in rainfed agriculture—Problems and prospects of fertilizer use in development of arid and semi-arid areas, *Fertil News*, **26**(11) (1981) 2-7.
34. Kanwar J S, Virmani S M & Kampen J, Management of Vertisols—ICRISAT Experience. *Symposium on Vertisols, 12th International Congress of Soil Science*, New Delhi, Vol 3, 1982, 94-120.
35. Kanwar J S, Agriculture and human environments, *Transactions of the Cost Working Group on Human Environment* (Bhabha Atomic Research Centre, Bombay) January 1971, 105-12.
36. Kanwar J S, Soil and water—The human environments, Committee on Human Environment, New Delhi, January 1971.
37. Kanwar J S, Use and conservation of the biosphere, *Indian J agric Sci*, **41**(2) (1971) 182-83.
38. Kanwar J S, Fertilizers and environmental pollution—Indian case analysed, *Indian Fmg*, **22**(9) (1972) 5-7; 19.
39. Kanwar J S, Land and soil environment programme at ICRISAT, *Soil Policy Committee Meeting*, FAO/UNEP, February 1981.
40. Kanwar J S, Save the soil—Save the nation, in *Education for environmental planning and coordination*, edited by Desh Bandhu and N L Ramanathan (Indian Environmental Society, New Delhi) 1982, 67-84.
41. Kanwar J S, Managing soil resources to meet the challenges to mankind, *Presidential Address, 12th International Congress of Soil Science*, New Delhi, Plenary Session Papers, Vol 1, 1982, 1-46.



## S Kedharnath

My research work on plants began in 1943, when I joined the Sugarcane Breeding Station (now Sugarcane Breeding Institute) at Coimbatore and investigated under the guidance of Dr N Parthasarathy, the cytogenetics of the intergeneric hybrids resulting from the cross between *Saccharum officinarum* and *Sclerostachya fusca*. In 1946, I joined the Botany Division (now Genetics Division) of the Indian Agricultural Research Institute, New Delhi, and worked on the cytogenetics of colchicine-induced autotetraploids of *Sesamum indicum* and interespecific hybrids of sesame, *Sesamum indicum* and *Sesamum prostratum*, the derived amphidiploid, sesquidiploid and its progeny in an attempt to evolve strains resistant to the caterpillar *Antigastra catalaunalis* and at the same time capable of giving high oil yield. My interest later shifted to breeding of linseed for oil content and good drying quality. I studied the effect of morphological grouping of the Indo-Gangetic alluvial types and the Peninsular types grown in the Deccan on the correlation coefficient of (i) yield of seed and yield components, and (ii) yield of oil and oil quality. An understanding of these basic relationships was considered essential to put linseed breeding on a sound scientific footing. To facilitate screening of a large population of segregating material for the selection of desirable types for oil quality, a rapid

method for determining oil quality was developed by working out the correlation between iodine value and refractive index of the oil expressed from a small sample of a few seeds.

During 1955-1958, I was on study leave in USA and worked in the Genetics Division of the University of Wisconsin at Madison under the guidance of Prof. R A Brink. My research work here was on mutable loci and transposable elements associated with such loci. The experimental material used was maize. The allele for variegated pericarp in maize had been earlier shown to be a dual structure. One component is considered to be the gene for self-red pericarp, *prr*, located near the mid region of the short arm of chromosome 1. The other component has been termed modulator (MP). It was found that when the modulator is present at the 'P' locus in conjunction with the *prr* gene, it suppresses the pigment producing action of the latter. Thus, *prr-MP* combination as an allele of P, conditions colourless pericarp and cob in the absence of mutation. This allele is unstable and mutates to red frequently and irregularly in somatic tissue. Mutation from colourless to red involves an extraordinary type of change, the nature of which was first discovered by the Nobel Laureate Barbara Mc Clintock in her classical study in maize

---

Formerly, Director, Kerala Forest Research Institute, Peechi-680653; Residence : 5, First Cross Road, Ramalinga Nagar, Layout V, K K Pudur Post, Coimbatore-641038.

of two transposable elements—dissociator (Ds) and activator (Ac). Ac and Ds were found to be transposable from one position in the genome to another. Such transposable elements have also been referred to as controlling elements. My investigations on the controlling element Mp gave evidence that Mp is a unitary germinal component.

From 1951 to mid-1955, I worked as a wheat breeder under the guidance of Dr B P Pal and was in charge of breeding work on early wheats at Pusa (Bihar) for high yield, good grain character and resistance to brown and stem rusts. Some interesting crosses were made and selections carried out from the segregating population under artificial condition of epiphytotics of the races of brown and stem rusts.

In 1960, I moved to the Forest Research Institute, Dehradun to head the newly created Genetics Branch and to handle forest tree breeding work. For the last nearly 25 years I have been actively engaged in this fascinating field of work. After my retirement from the Forest Research Institute, I joined the Kerala Forest Research Institute, Peechi, an autonomous research institute as its Director and worked there till March 1986. I also held additional charge of the Division of Genetics and continued work on breeding of forest trees and started some new experiments. Forest trees in general have a long generation period and they have not been domesticated so far. They are indeed wild populations and there is plenty of scope for improving them by selection and breeding. I concentrated on a few selected species—teak (*Tectona grandis*), chir pine (*Pinus roxburghii*) and 2 species of eucalyptus (*Eucalyptus tereticornis* and *E. grandis*). Some effort was also put in to study the radiosensitivity

of some selected species to physical and chemical mutagenesis. The major work carried out is discussed below.

### Teak (*Tectona grandis*)

With the objective of effecting genetic improvement in this species, first its chromosome number was determined. The somatic chromosome number was found to be  $2n = 36$ . Meiosis of pollen mother cells at metaphase-I showed regularly 18 bivalents. Criteria for the selection of outstanding individual trees which exhibit one or more desirable traits, such as superior height growth and diameter, narrow crown, active leader, long clear bole, good cylindrical bole without fluting, absence of epicormic shoots, freedom from important insect pests, such as defoliater, *Hyblaea puera* and leaf skeletoniser, *Eutectona machaeralis*, were fixed. More than 800 plus trees have been selected so far in the different states. Most of these have been inspected and approved as 'plus trees' and are being used for establishing clonal seed orchards. The first experimental clonal seed orchard for teak was established by me and my colleagues at New Forest Campus, Dehradun. There is general evidence from the data collected so far of the presence of genetic variation for most of the characters referred to above. More recently, in certain regions of Kerala, an insect, *Cossus cadambae*, which was hitherto innocuous, has attained a pest status and is killing mature trees. It is being investigated by us in detail.

Grafting/budding techniques were studied for use in the field as well as for bench grafting where naked stumps (root-shoot cutting about a year old) are used as stocks and buds from plus trees are budded at the collar region and planted in



soil contained in polypots. After about 3 weeks, the buds sprout. The 20-clone orchard established at New Forest has yielded valuable information on variation in flowering, susceptibility/resistance to two leaf infecting fungi (though economically not important), *Olivaea tectonae* and *Caldariomyces tectonae*. Variations in wood characters were studied using material from a provenance experiment and this brought to light geographic and individual tree variations in fibre length. The seed orchard in New Forest gave seeds in the sixth year; 80% of these germinated against 20% in the case of seeds collected from the normal trees.

*Encalyptus tereticornis* and *E. grandis*: These species are now being raised in large plantations in the Nilgiri hills. Genetic improvement work on them was, therefore, taken up. Plus trees have been selected and progeny trials have been established to obtain information on various genetic parameters. The evaluation of a progeny trial of *E. tereticornis* showed that there was large genetic variation among the families for height and diameter at the ages of 3 and 4 years. Correlation for height and diameter over different years showed that the second year growth can be used for reliable prediction of future performance in both height and diameter. As far as heritability is concerned, the value obtained at ages 3 and 4 years for height was rather low, being 0.26 and 0.25 and 0.17 for diameter at ages 3 and 4 years.

Similarly, in the case of *E. grandis*, evaluation of the progeny trial showed sufficient genetic variation in plant height. The heritability estimate was 19-20% for 3 year old trees and 34% for 4 year old ones.

### Chir Pine (*Pinus roxburghii*)

Nine provenances of this species have been recognized based on growth characteristics. Considerable variation in oleoresin yield was noticed between the provenances and within the provenances in the trees growing at New Forest, Dehradun. A number of plus trees were selected for use in genetic improvement work. Clonal propagation techniques were worked out. Cleft grafting and air layering gave very high success rate. The karyotype of the chromosomes of this species was studied and compared with those of some other pine species native to India. Variation in growth, morphological characters and some wood characters were studied using as experimental material a progeny test established at New Forest, Dehradun from open pollinated seed. Heritability values and correlation between different characters were also worked out.

### Induced Mutagenesis

The response of air dried seeds of 10 forest tree species—*Abies pindrow*, *Picea smithiana*, *Pinus wallichiana*, *Pinus roxburghii*, *Pinus kesiya*, *Shorea robusta*, *S. talura*, *Dalbergia sissoo*, *Dalbergia latifolia* and *Santalum album* to different doses of acute gamma irradiation was assessed. The gymnosperm species appeared more radiosensitive compared to the angiosperm species. One interesting observation during this investigation was the high radiosensitivity, very close to that of gymnosperm species, exhibited by the two species of *Shorea* used in the experiment. This is presumably due to the very high moisture content of the air dried seeds.

Studies of the response of seeds of three tree species, *Pinus roxburghii*, *Dalbergia sissoo* and *Dalbergia latifolia*, to treatment with the chemical mutagen, ethyl methane sulphonate (EMS) showed that the two angiosperm species, *Dalbergia latifolia* and *D. sissoo*, were more sensitive than the conifer species, *P. roxburghii*. While in the case of *D. latifolia* the pH of the treatment medium had no influence, in the case of *P. roxburghii* and *Dalbergia sissoo*, the seeds were more sensitive at pH 9 than at pH 5 in that they needed less concentration of EMS to give 50% reduction in germination.

With ionizing radiations, a dose which restricts survival to 50% (LD 50) or growth to 50% (GR 50) is a good treatment. In the case of chemical mutagens, where the determination of (LD 50) is complicated by the difficulty of determining effective termination of treatment, the practical way is to use a graded series of dilutions or treatment durations. The temperature during the treatment is kept constant, because it has a profound effect on the action of the mutagenic chemicals. A dose and treatment regime which reduces the growth attained in a fixed period to 50% of the control is determined for each species.

### Selected Publications

1. Kedharnath S & Brink R A, Transposition and stability of 'modulator' in maize, *Genetics*, **43** (1958), 695-704.
2. Kedharnath S, Ramanujam S & Joshi A B, Chromosome pairing in two sesquidiploid hybrids and its bearing on genome relationship in the genus *Sesamum*, *Indian J. Genet.*, **19** (1959), 201-9.
3. Kedharnath S, Joshi A B & Batcha M G B R, Correlation studies in *Linum usitatissimum*. II—Effect of morphological grouping of types on the correlation coefficients relating to yield and some of the components of yield, *Indian J Genet*, **20** (1960), 58-68.
4. Kedharnath S, Joshi A B & Batcha M G B R, Correlation studies in *Linum usitatissimum*. III—Effect of morphological grouping of types on the correlation coefficient relating to oil content and oil quality, *Indian J Genet*, **20** (1960), 69-78.
5. Kedharnath S & Raizada M B, The role of cytology in forest genetics research, *Proc 5th World Forestry Congress*, **2** (1960), 757-59.
6. Kedharnath S & Raizada M B, Forest genetics and tree breeding, *Proc. 10th Silvi Conf*, Dehradun, **1** (1961), 203-14.
7. Kedharnath S & Matthews J D, Improvement of teak by selection and breeding, *Indian Forester*, **88** (1962), 277-84.
8. Kedharnath S, Chacko V J, Gupta S K & Matthews J D, Geographic and individual tree variation in some wood characters of teak (*Tectona grandis* L.f), *Silvae Genetica*, **12** (1963), 181-86.
9. Kedharnath S & Venkatesh C S, Genetic improvement of *Eucalyptus* in India, *Silvae Genetica*, **14** (1965), 155-59.
10. Kedharnath S, Tree improvement—Its impact on man-made forests, *Proc FAO World Symposium on Man-made Forests*, Australia, **1** (1957), 435-61.
11. Kedharnath S, Rawat M S & Chauhan V S, Early growth performance of 20 clones of teak (*Tectona grandis* L.F) in a seed orchard, *Proc Seminar-cum-workshop on Genetic Improvement of Forest Tree Seeds in India*, (1970), 86-89.
12. Kedharnath S, Chetty C K R & Upadhaya L P, Estimation of risk of reversal of arms and order of chromosomes in karyotype analysis of *Pinus roxburghii* Sarg, *Indian Forester*, **96** (1970), 812-16.
13. Kedharnath S, The long-range outlook for production of rust resistant trees by induced mutagenesis in Symp. on Biology of rust resistance in forest trees. *USDA Forest Service Misc Publ*, 1221 (1972), 551-59.
14. Kedharnath S, Studies on the response of some selected tree species to chemical and physical mutagens, *Proc IUFRO/SABRAO Joint Symp for Forest Tree Breeding*, Tokyo, D. 10(V), 1972 1-8.
15. Kedharnath S, & Upadhaya L P, Sensitivity of seeds of Chir pine of different seed origin to acute gamma irradiation, *Indian J Genet*, **34A** (1974), 393-99.



16. Kedharnath S & Lakshmikantham D, Effect of acute gamma irradiation on two species of Shorea, *Indian J Genet*, **34A** (1974) 401-7.
17. Kedharnath S & Kapoor M L, Field grafting trials with Caribbean pine, *Indian Forester*, **102**(6) (1976), 279-82.
18. Kedharnath S, Kapoor M L & Vakshasya R K, Field grafting studies in *Pinus patula*, *Indian Forester*, **103**(6) (1977), 397-402.
19. Kedharnath S & Vakshasya R K, Estimates of components of variance, heritability and correlation among some growth parameters in *Eucalyptus tereticornis*, *Proc III World Consultation on Forest Tree Breeding*, Canberra, Australia, **2** (1977), 667-76.
20. Kedharnath S, Kapoor M L & Vakshasya R K, A note on field grafting in Chir pine, *Indian Forester*, **105**(4) (1979), 301-4.
21. Kedharnath S, Plus tree selection—A tool in forest tree improvement in *Proc natn Symp on Improvement of Forest Biomass*, edited by P K Khosla (Indian Tree Scientists, Solan) 1982, 13-20.
22. Kedharnath S, Genetic variation and heritability of juvenile height growth in *Eucalyptus grandis*, *J Tree Sci*, **1** (1982), 46-49.
23. Kedharnath S, Genetics and forest tree breeding, in *Genetical research in India*, edited by P L Jaiswal and A M Wadhwani (ICAR, New Delhi) 1983, 181-90.

## Abrar M Khan

Khan started his research work in 1942 in the Department of Botany, Aligarh Muslim University, Aligarh, under the guidance of Dr Rafiq A Khan. He later joined the Department of Plant Pathology at the University of Minnesota, which at that time under the stewardship of Prof. E C Stakman had developed into the Mecca of plant pathological research. On his return to Aligarh in 1950, Khan initiated research in plant pathology where several distinguished future plant pathologists and nematologists of the country received their initial training. The research work carried out by him falls into two broad categories: (a) Basic and applied aspects of fungal plant pathogens; and (b) Taxonomy, ecology, host-parasite relationship, population dynamics and control of economically important plant parasitic nematodes.

During the course of studies for his doctorate degree at the University of Minnesota, Khan found that deficiency of bivalent ions, viz., calcium and magnesium, predisposed leguminous plants to the attack of *Rhizoctonia solani* Kuhn. Part of his thesis on this problem was published by the University of Minnesota in the form of Experimental Station Bulletin; the rest he published himself.

Khan and his coworkers initiated work on whip smut of sugarcane, *Ustilago*

*scitaminea*, and concluded that there were two flushes of smut, one in April-May and the other in October. Chlamodospores on germination produced promycelium having four cells, each one producing sporidium with a single haploid nucleus and from the point of view of sex, the segregation was bipolar. Studies based on pathogenicity revealed the involvement of a single species of smut fungus and not two as suggested by Mundukar and Thirumalachar for the causation of disease.

From the work carried out on anthracnose disease of Sorghum, *Colletotrichum graminocolum* (Ces) Wils, it was established that cultivars having higher sugar content were more susceptible to the disease than others. It was also shown that a closely related species, *Colletotrichum falcatum* Went, the causal organism of red rot of sugarcane, was capable of causing seedling blight of sorghum seedlings, although it did not produce anthracnose on this host.

Another fungal disease which attracted attention was powdery mildew of cucurbits, which in the Indo-Gangetic plains of India takes a heavy toll of the crop each year. It was found that two species, viz., *Erysiphe cichoracearum* DC and *Sphaerotheca fuliginea* (Schlecht) Poll, are involved in the causation of the disease and both have large numbers of common hosts. It was found that they



could be distinguished in the absence of perfect stage on host specificity—the former infects *Coccinia cordifolia* and not *Lagenaria leucantha* and the latter *L. leucantha* and not *C. cordifolia*.

Studies on varietal resistance revealed that two cultivars out of five of *C. vulgaris* var. *fistulosus*; one out of 28 of *C. melo*; nine out of 16 of *C. melo* var. *utilissimus*; and one out of 20 of *C. sativus* were resistant to the attack of *S. fuliginea*. Similar results were obtained when different cultivars were inoculated with *Erysiphe cichoracearum*.

Although the two fungi rarely produce perfect stage in the field, when inoculated plants were grown in growth chambers, production of perfect stage was favoured by low temperature and low relative humidity. These studies provided scientific explanation for non-production of perithecia in nature, as these conditions do not prevail during the period when cucurbits are grown in North India.

In a symposium organized by the Indian National Science Academy, based on his observations on the availability of susceptible cultivars and the environmental conditions which prevailed in Kashmir Valley, Khan, as early as 1955, pointed out the potentiality of apple scab fungus *Venturia inaequalis*, in the valley.

Khan became interested in phytone-matology under fortuitous circumstances in the late fifties. In one of his orchards, papaya seedlings exhibiting unthrifty growth were full of galls of varying sizes of root-knot nematode, *Meloidogyne incognita*. He and his students initiated work on several aspects, such as host range, factors influencing hatching; and attractiveness of excised roots to root-knot

larvae. They undertook work on nematode taxonomy and described about 100 species of nematodes. Out of them, *Heterodera mothi* Khan and Husain, 1965, was the most important, because till then it was believed that *Heterodera* spp. are restricted to cooler regions of the world.

Random surveys for the study of nematode fauna infesting a variety of crops were conducted in several states. The important problems identified were the association of root-knot nematodes with vegetables; reniform nematodes, *Rotylenchulus reniformis* on pulses and oil crops; *Tylenchus semipenetrans* with citrus; and *Hirschmanniella* species with rice and lesser millets.

A large number of varieties of vegetables and other crops were screened against several nematodes, specially against root-knot, reniform nematode. Sources for resistance were identified for different crops. The temperature, moisture and structure of the soils influence profoundly the increase in nematode population. In perennial plants, each period of root growth increased the population of nematodes in addition to the above factors.

Okra, tomato and egg plants infested with root-knot suffered from potassium deficiency symptoms, specially when they were exposed to higher initial inoculum densities. It was observed that root-knot infection did not impair the absorptive capacity of K ions, as the amount of potassium in healthy plants remained the same. In the diseased plants, on the other hand, K ions were confined mostly to roots, as if the transport of these ions was disturbed because of the damage of vascular tissue caused by nematode invasion.

For the control of nematodes, several chemicals, DD, Vapam, Nemaphos, Solvirex, Thimet 10G, Rogor G, Basamid and VC-13, were tested. All of them proved to be effective for the control of nematodes to varying degrees. In view of the high cost-return ratio and the likely pollution hazards, the efficacy of organic amendments in the form of oilcakes was tried. By and large, oilcakes as well as deoiled cakes proved to be as effective as the above pesticides in mitigating the ill-effects of nematode infection. In field tests, when the soil was amended with oilcakes, the population of plant parasitic nematodes and pathogenic fungi was reduced, whereas the population of saprozoic nematodes and saprophytic fungi increased.

Root-knot development was arrested in the seedlings dipped in the water soluble fraction of the oilcakes. In treated seedlings, there was a greater concentration of phenolic compounds. Root-knot also failed to develop in seedlings dipped in phenolic and related compounds. Phenolics having OH group at *para* and *ortho* positions exhibited greater nematicidal activity than compounds having OH group at *meta* position. Evidence was also provided that oilcakes killed nematodes on the one hand and provided a certain degree of resistance to plants on the other.

Interactions between pathogenic fungi and nematodes and between nematodes and nematodes were also studied. These combinations resulted in a loss that was more than additive.

The first nematology course financed by the Rockefeller Foundation, the Southeast Asia Postgraduate Nematology Course jointly organized by the International

Agriculture Centre, Wageningen, the Netherlands, Indian Agricultural Research Institute, New Delhi and the Botany Department, Aligarh Muslim University, Aligarh, and the establishment of Nematological Society of India were initiated through Khan's pioneering efforts. In recognition of his contributions, Khan was awarded the Rafi Ahmed Kidwai Memorial Award in 1974 by ICAR and the certificate of honour by the Haryana Agricultural University in 1984. He was elected the first President of the Nematological Society of India in 1969-70; President, *Acta Botanica Indica* in 1977 and President, Indian Science Congress (Section of Agricultural Sciences) in 1984. He is a fellow of several learned societies, including the Indian National Science Academy. Khan has published more than 300 research papers.

### Selected Publications

1. Kernkamp M F, de Leeuw D J, Chen S M, Ortega B C, Tsiang C T & Khan A M. Investigation on physiological specialization and parasitism of *Rhizoctonia solani* Kuhn. *Univ Minn Agric Exp Sta Tech Bull* (1952) 200.
2. Khan A M & Asad Ahmad, Factors influencing larval hatching in the root-knot nematodes, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood 1949 and *M. javanica* (Treub, 1885), (Chitwood, 1949). I-Effect of temperature and hydrogen-ion concentration, *Indian Phytopath*, **XVII** (1964) 98-101.
3. Khan A M & Israr Husain S, A new genus and six new species of nematodes from India belonging to the family Neotylenchidae with amendment of the sub-family Ecphyadorphorinae, *Proc helm Soc, Wash*, **32**(1) (1965) 7-15.
4. Khan A M, *Heterodera mothi* n sp (Tylenchida: Heteroderidae) parasitizing *Cyperus rotundus* L. at Aligarh, UP, India, *Nematologica*, **11**(2) (1965) 168-72.
5. Khan A M & Israr Husain S, Four new species of the genus *Diphtherophora* de Man, 1880



- (Nematoda: Diptherophoridae) with a key to the species of the genus, *Proc helm Soc, Wash*, **32**(2) (1965) 186-91.
6. Khan A M & Israr Husain S, A new sub-family, a new sub-genus and eight new species of nematodes from India belonging in the family Tylenchidae, *Proc helm Soc, Wash*, **34**(2) (1967) 175-86.
  7. Khan A M & Israr Husain S, On the status of the genera of the superfamily Aphelenchoides (Fuchs) 1927, Thorne, 1949 with the description of six new species of nematodes from India, *Proc helm Soc, Wash*, **34**(2) (1967) 167-74.
  8. Khan A M, Influence of organic amendments on plant parasitic nematodes, *Proc. International Congress of Plant Pathology*, London, 1968.
  9. Khan A M, *Studies on plant parasitic nematodes associated with vegetable crops in Uttar Pradesh* (Aligarh Muslim University) 1971.
  10. Khan A M, Haque Q A & Saxena S K, Studies on the effect of different levels of certain elements on the development of root-knot. I. Effect of NPK levels on growth of okra and root-knot development, *Indian J Nematol*, **2** (1972) 35-41.
  11. Khan A M, Rashid A & Khan F A, Plant parasitic nematodes associated with fruits, vegetables, cereals and other crops in North India. I. Uttar Pradesh, *Indian J Nematol*, **3** (1973) 8-23.
  12. Khan A M, Haque Q A & Sexena S K, Studies on the effect of different levels of certain elements on the development of root-knot. II. Effect of different levels of potassium on growth and root-knot development on okra, eggplant and tomato, *Indian J Nematol*, **4** (1974) 25-30.
  13. Khan A M, *Studies on powdery mildew resistance in cucurbits* (Aligarh Muslim University, Aligarh) 1976, pp 100.
  14. Khan A M, *Control of diseases caused by nematodes by the application of oilcakes* (Aligarh Muslim University, Aligarh) 1973, pp 92.
  15. Khan A M, Singh R P & Saxena S K, Effect of salinity, alkalinity, phosphate and organic matter on the movement of nematicides in soil using thin-layer-chromatography, *Nematol Medit*, **9** (1981) 139-43.

## Gurdev S Khush

During the 28 years of my research career, I have carried out researches on three important food crops, aimed at understanding the genetic architecture of these crop species. During the last 18 years, I have directed my research efforts towards developing high yielding varieties of rice, which is the most important food crop in the world.

For my PhD thesis research, I investigated the cytogenetic relationships of species of genus *Secale* to which cultivated rye, *Secale cereale*, belongs. I showed for the first time that chromosomal rearrangements have played a major role in the differentiation of the species of the genus, as all the cultivated and wild species of the genus differ by a series of translocations. This work is considered to represent an authoritative study of the cytotaxonomy of this important genus. This work is considered to represent an authoritative study of the cytotaxonomy of this important genus. This study also threw light on the origin of cultivated rye and it was shown that gene exchange between cultivated rye and all the wild species, except one, was possible.

During August 1960-July 1967, I carried out basic researches on the nature of tomato genome, another crop of worldwide importance. These studies were aimed at (i) associating linkage groups of

tomato with the respective chromosomes, (ii) determining the orientation of the linkage groups and mapping the centromere positions on the linkage maps, and (iii) mapping the unlocated and unmapped genes. Two techniques were employed for this purpose.

The induced deficiency technique involved irradiating the pollen from plants with normal alleles and using this pollen for pollinating the plants homozygous for mutant alleles at one or more loci; progenies sired by these pollinations were searched for pseudodominants, e.g. plants showing the mutant traits. These exceptional progenies were examined cytologically at pachytene and the missing chromosome segments were determined. By studying a series of overlapping deficiencies, mutant genes were located to small chromosome segments. A total of 32 genes were located on 12 chromosomes of tomato. After 2 or 3 genes had been located on the respective chromosome arms, it was possible to determine the orientation of the linkage map, e.g., which genes are on the long arm and which are on the short arm of the chromosome; in several cases it was possible to map the exact centromere position on the linkage map.

Two primary and 25 tertiary monosomics as well as five monoisodi-



somics were identified among the progenies obtained from the irradiated pollen. It was demonstrated that the longer arms of tomato chromosomes tolerate only small deficiencies, while the shorter arms are comparatively less important for the survival of the sporophyte. Thus, deficiencies of one entire short arm or even two short arms (as in the case of primary or tertiary monosomics) may be tolerated. The total length of the missing short arms may be as much as  $24\mu$ , but in the case of long arms, deficiencies longer than  $7\mu$  were never tolerated. This differential tolerance for deficiencies of long and short arms was demonstrated for the first time in any organism.

The second technique employed for determining the arm location of the marker genes and for centromere mapping was the modified ratio technique using trisomics. For this purpose, a series of secondary, tertiary and telotrisomics were established. Several compensating trisomics were also established, one of which had never been reported before. In this trisomic, two isochromosomes, one for the long arm and the other for the short arm, replaced one normal chromosome 7. As expected, the plant was indistinguishable from the primary trisomic for chromosome 7, but the breeding behaviour was entirely different. All these trisomics were utilized in linkage mapping and 34 genes were located to the respective chromosome arms. The cytogenetics of the secondary trisomics was investigated for the first time.

As a result of extensive investigations using the induced deficiency technique and the trisomic technique, all the linkage groups were associated with their respective chromosomes; all the

centromeres were mapped, 129 genes were delimited to the respective chromosome arms and orientations of all the linkage groups were determined. These investigations were reported in a series of 22 papers published in major international journals; two of them appeared as chapters in books. Several of the publications are considered authoritative in the field and are included in the list of required reading materials for students taking cytogenetic courses at many universities. As a result of these investigations, tomato is one of the best genetically studied species.

The investigation on aneuploids of tomato and other crop and animal species were reviewed in the book "*Cytogenetics of Aneuploids*" (Academic Press, New York, 1973).

I became interested in applied genetics after moving to the International Rice Research Institute in 1967 and have played a leading role in the development of high yielding, disease and insect resistant varieties of rice. This work has resulted in the release of 50 high yielding varieties of rice in different countries. Twenty two breeding lines developed by me have been recommended as varieties by the Philippine Seedboard. These varieties are planted in 80% of the rice area in the Philippines and have been widely adopted in the other rice growing countries of Asia, Africa and Latin America. Approximately 30 million ha of rice land in the world is under varieties developed under my leadership. One of these varieties, IR36, is alone planted in more than 10 million ha of rice land. No other variety of rice or of any other food crop has been planted in that large area before. IR36 is the most widely planted variety of rice in the Philippines,

Indonesia and Vietnam. As a result of its wide-scale adoption, Philippines became a rice exporting country and Indonesia and Vietnam have become self-sufficient. The insect resistance of IR36 alone saves the rice farmers in the developing world untold millions per year, money that would otherwise have gone to purchase petroleum-based insecticides. The quinquennial review mission composed of 12 international experts which reviewed the IRRI research and training programmes on behalf of the Technical Advisory Committee of CGIAR in 1981 has pointed out, "The impact of IR36 alone would more than justify the investment in IRRI since its establishment 21 years ago."

I have also carried out extensive researches on the basic genetics of rice plant. I have developed a research programme on the genetics of host-parasite interactions. This programme has led to the identification of numerous genes for resistance to major diseases and insects of rice. These genes have been incorporated into the improved germplasm to develop varieties with multiple resistance. National rice improvement programmes are also making extensive use of these genes. As an example, *Oryza nivara*, a wild relative of rice, is the only known source of resistance to grassy stunt virus. Genetic analysis revealed that it has one dominant gene for resistance to this disease. This gene was transferred to *O. sativa* by backcrossing and incorporated into 16 improved varieties like IR36. Scientists in other rice growing countries are also using the same gene for developing resistant varieties. This research has been reported in more than 40 research papers.

Soon after my arrival at IRRI, I established a complete series of primary trisomics of rice. Extra chromosome of each trisomic was identified at pachytene stage of meiosis and their breeding behaviour was studied. Segregation of 22 marker genes in the primary trisomic progenies was investigated. The results obtained established the association of linkage groups with the cytologically identifiable chromosomes for the first time.

I plan to continue the research efforts to breed new varieties of rice with even higher yield, resistance to diseases and insects, superior grain quality, shorter growth duration and with adaptation to varied rice growing conditions. The emerging techniques of biotechnology will be utilized in rice improvement work. The programme on rice genetics will be further strengthened to identify additional genes for resistance to diseases and insects. The research programme on trisomic inheritance and linkage mapping will be expanded to develop the chromosome map of rice.

### Selected Publications

1. Khush G S, *Cytogenetics of aneuploids* (Academic Press, New York) 1973, pp 301.
2. Khush G S, Rice, in *Handbook of genetics*, Vol 2, edited by R C King (Plenum Press, New York) 1975, 31-58.
3. Khush K S, Breeding for resistance in rice, in *The Genetic Basis of Epidemics in Agriculture*, Ann NY Acad Sci, **287** (1976) 296-308.
4. Khush G S, Disease and insect resistance in rice, *Recent Adv Agron*, **29** (1977) 265-341.
5. Khush G S, Breeding for multiple disease and insect resistance in rice, in *Biology and breeding resistance to arthropods and pathogens in agricultural plants*, edited by M K Harris (Texas A & M University, College Station, Texas) 1979, 341-54.
6. Khush G S, Cytogenetics and evolutionary studies in Secale. II. Interrelationships of the wild species, *Evolution*, **16** (1962) 848-96.



7. Khush G S & Rick C M, The origin, identification and cytogenetic behaviour of tomato monosomics, *Chromosoma*, **18** (1966) 407-20.
8. Khush G S & Rick C M, Novel compensating trisomics of the tomato: Cytogenetics, monosomic analysis, and other applications, *Genetics*, **56** (1967) 297-307.
9. Khush G S & Rick C M, Tomato tertiary trisomics: Origin, identification, morphology, and use in determining position of centromeres and arm location of markers, *Can J Genet Cytol*, **9** (1967) 610-31.
10. Khush G S & Rick C M, Tomato telotrisomics: Origin, identification, and use in linkage mapping, *Cytologia*, **33** (1968) 137-48.
11. Khush G S & Rick C M, Cytogenetic analysis of tomato genome by means of induced deficiencies, *Chromosoma*, **23** (1968) 452-84.
12. Khush G S & Rick C M, Tomato secondary trisomics: Origin, identification, morphology and use in cytogenetic analysis of the genome, *Heredity*, **24** (1969) 129-46.
13. Khush G S & Ling K C, Inheritance of resistance to grassy stunt virus and its vector in rice, *J Hered*, **65** (1974) 135-37.
14. Khush G S, Ling K C, Aquino R C & Aguiro M V, Breeding for resistance to grassy stunt in rice, *Proc 3rd Int Cong SABRAO*, Canberra, Australia, *Plant Breeding Papers*, **14(b)** (1977) 3-9.
15. Sidhu G S & Khush G S, Dominance reversal of a bacterial blight resistance gene in some varieties of rice, *Phytopathology*, **67** (1977) 461-63.
16. Hernandez J E & Khush G S, Genetics of resistance to whitebacked planthopper in some rice varieties, *Oryza*, **18** (1981) 44-50.
17. Khush G S, Singh R J, Sur S C & Librojo A L, Primary trisomics of rice: Origin, morphology, cytology and use in linkage mapping, *Genetics*, **107** (1984) 141-63.

## K B Lal\*

I started research on the cytoplasmic inclusions in the eggs of certain Indian tortoises and snakes, involving largely mitochondria and Golgi bodies. Subsequently, I shifted to agricultural entomology and studied the systematics and biology, including parasites, of the Homopterous family of Scottish Psyllidae at Edinburgh University.

On my return to India, I studied the bionomics of a serious cotton pest, *Empoasca devastans* Dist., on some varieties of cotton in the Punjab at Lyalpur (now Faislabad in Pakistan). Later, another pest of cotton, the cotton leaf roller, *Sylepta derogata* Fabr., was studied for its relative incidence on, hirsutum cottons in UP and its control.

Some taxonomic studies of parasitic hymenoptera were conducted at New Delhi. The Eulophid genus, *Azotus*, Chalcidoidea, was recorded for the first time from India, its new species *Delhiensis* having been bred from a white fly pest of sugarcane, which was also a new record, because the parasite till then had been

known to attack scale insects only. Seven new species of hymenopterous parasites were described; they were found to attack mostly sugarcane pests in different parts of India. Another study concerned an investigation into the identification of two Braconid species, *Bracon hebetor* and *B. brevicornis*.

Subsequent research activities largely involved the guidance of research conducted by others and chiefly concerned the biology and control of pests of cotton, sugarcane and fruit trees and control of field rats.

Though not exactly research, a notable scientific activity was the initiation of a plant protection service in Uttar Pradesh in 1947, which was claimed to be the first of its kind in India, and subsequently to catalyze the development of plant protection work in the country (1953-62) and still later in some countries of the Near East under an assignment of the Food and Agriculture Organization of the United Nations.

---

Chandra Bhawan, Civil Lines, Azamgarh, UP.

\*Since deceased.



## G Madhava Reddy

The major thrust in our laboratory is on understanding gene regulation, gene substrate and gene-enzyme relationship in anthocyanin biosynthesis in maize; evolving dwarf types in rice; genetic and physiological studies of dwarfs and semidwarfs; mutation breeding of rice; improvement of nutritional quality and protein content; genetic and biochemical basis of disease resistance in rice; chemotaxonomic studies of wild rices; and utilization of plant cell and tissue culture techniques in genetic manipulation of economically important crop plants like cereals (rice, maize, triticale), legumes (peanut, pigeonpea, chickpea), oilseeds (safflower, sunflower) and fruit crops (mango, grapes, guava).

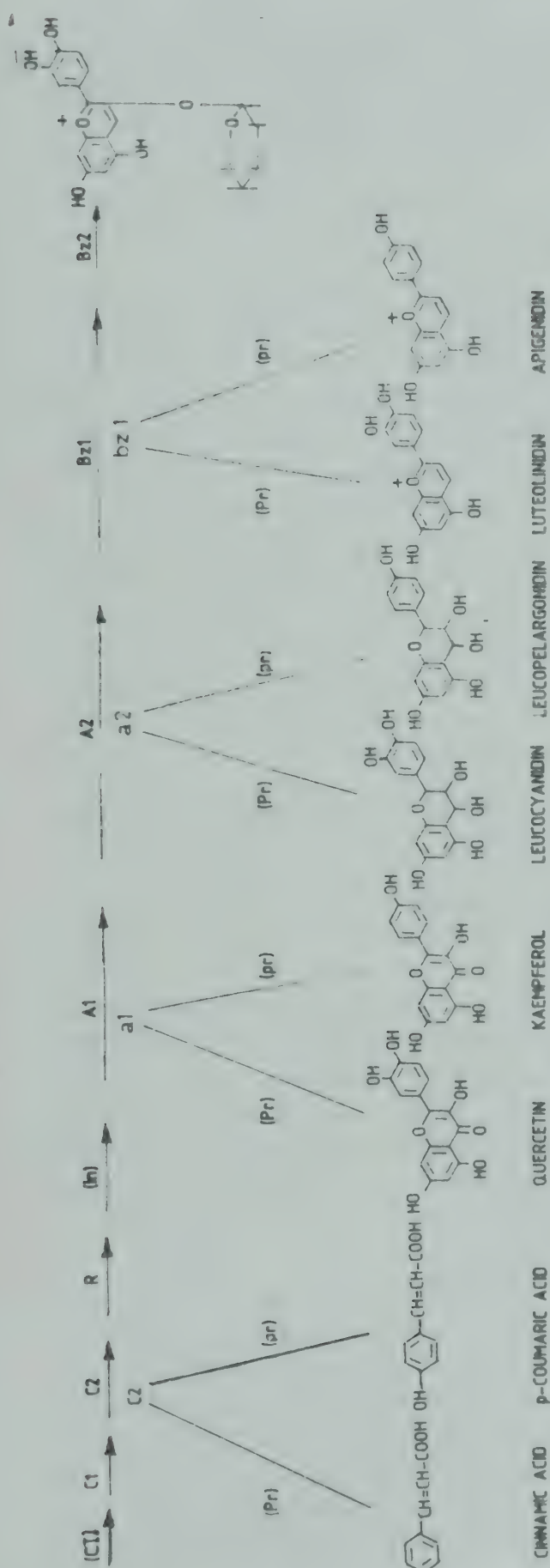
### Gene Action and Gene Regulation Studies in Maize

Anthocyanin pigment system in maize with well established genetic markers can be exploited for the understanding of the mechanism of gene action and gene regulation, especially in view of the modern biochemical methods with which the gene controlled end products can be identified. The complementary genes *A1*, *A2*, *C1*, *C2* and *R* must be present in dominant condition at least in one dose for the production of anthocyanin in aleurone tissue, the outermost layer of the triploid endosperm. If any one of these is homozygous recessive or if dominant *C1* is

present, colourless aleurone results. Recessive *bz1* and *bz2* result in bronze pigment, *pr* in red pigment and *in* (intensifier) enhances the anthocyanin and related pigments in aleurone. Direct evidence for gene action sequence came from the inter-tissue complementation studies with fresh aleurone tissue (Fig. 1).

Fresh excised colourless aleurone tissue (20-25 days old) of single recessive genotypes when pressed together in pairs, two at a time, resulted in pigment synthesis in only one tissue of the pair, the receiver. For example, in *a1* aleurone tissue of *a1* : *a2* pairs pigment synthesis was observed only in *a1* aleurone (receiver) due to the transfer of diffusible substances from the donor *a2* tissue. The pigment synthesis was unidirectional, although the donor-receiver relationship was interchangeable. The analysis of all possible paired combinations of different genotypes has led to the unidirectional gene action sequence for anthocyanin biosynthesis: (*C1*) - *C1* - *C2* - *R* - (*In*) - *A1* - *A2* - *Bz1* - *Bz2* cyanidin - 3 - glucoside.

The isolation and characterization of the accumulated substances by chromatographic, spectrophotometric and chemical tests led to the conclusion that double receiver *al Pr* accumulates quercetin; *al pr* accumulates kaempferol; *a2 Pr*, leucoanthocyanidin; *a2 pr*, leucopelargo-



**Fig. 1**—Gene controlled pathway for anthocyanin biosynthesis in maize

## Induced Dwarfs and Their Genetic Studies in Rice

Rice is the staple food for two thirds of the world population and accounts for almost half of the daily caloric intake, especially in the Asian countries. The various aspects on which work was done in this field include the recovery of fine grain mutants in IR-8 and other local cultivars, use of local cultivars to recover dwarfs and semidwarfs, identification of lines with superior protein content and distribution, and isolation of non-allelic dwarfing genes among induced dwarfs, and biochemical basis of speciation. In addition, certain basic aspects of mutagenesis too were covered.

Short statured mutants have played a significant role, especially in the development of non-lodging and high yielding rice varieties. The rapid spread of high yielding varieties like IR-8, Jaya, Sona, etc. with the same DGWG dwarfing genes introduces the danger of constantly exposing the same background to the adaptation of specific pathogens; the introduction of non-allelic dwarfing genes may help in avoiding genetic vulnerability. The allelic nature of five induced dwarfs recovered from Tellakattera was studied in relation to the DGWG dwarfing gene which demonstrated that these induced



dwarfs were non-allelic to the DGWG dwarfing gene.

### **Improvement of Nutritional Quality of Maize and Rice**

The opaque-2 gene which boosts the production of essential amino acids like lysine by 69-100% and of tryptophan by about 66% over the normal maize was incorporated into 11 Indian inbreds with a view to developing a composite hybrid variety of high protein quality. The half opaque recovered had better seed texture and high percentage of protein (12.6%), although there was a slight reduction in lysine content; the texture was superior to that of the control.

The miracle rice, IR-8, is one of the high yielding dwarf varieties which has revolutionized the yielding potential of rice crop. Mutation breeding of IR-8 has led to the recovery of five mutants with translucent kernels. Some of the fine grain mutants, viz., FG-12, FG-19 and FG-20, with medium slender grains excelled control in head rice recovery. The scented rices have an important place in consumer preference because of their desirable features, which include aroma, elongation of grain during cooking and medium amylose content. Studies on the essential amino acid profiles of 12 scented varieties and one non-scented variety revealed that scented varieties exhibit higher values for some of the essential amino acids. Since lipooxygenase, responsible for the production of off-flavours during storage, has lower activity in scented rices, it was suggested that scented rices possess better storage quality than nonscented rices.

Studies on protein contents of 124 induced grain type mutants and several

other local cultivars led to the identification of lines with deeper distribution and superior protein content (13.7%); these are being used in the rice improvement programmes.

### **Chemotaxonomic Studies on Wild Rices**

Phylogenetic relationships of certain species in the genus *Oryza* have been studied on the basis of biochemical traits, such as free amino acid and amino acid profiles of seed proteins to gain further insight into phylogenetic relationship. The amino acid profiles in seeds of 13 different species of *oryza*, including two cultivated rices, *O. glaberrima* and *O. sativa*, and the two major geographical races, *indica* and *japonica*, were studied; this work provided independent evidence to speciation, confirming the results of earlier cytogenetic studies.

### **Biochemical Studies of Gall-Midge Resistance**

Biochemical studies on gall-midge resistance were carried out with resistant and susceptible varieties. The aspects covered included their phenols, reducing and non-reducing sugars and peroxidase and esterase isozymes. The results obtained suggested an association of resistance with higher phenol content and the presence of a specific additional isozyme of peroxidase and esterase.

### **Cell and Tissue Culture Studies**

Several economically important crop plants, such as rice, maize, triticale, groundnut, pigeonpea, chickpea, safflower, sunflower and soyabean, besides certain fruit crops, have been established in culture and regenerated successfully from different plant parts like root internodes,

shoot, leaf, embryo, hypocotyl, epicotyl, anther, inflorescences, etc. Regenerated plantlets were grown to maturity in greenhouse to evaluate their agronomic potential and genetic variation for desirable characters.

In rice, plantlets were regenerated in long-term cultures even after 228 days from internodal callus and beyond 900 days from embryo calli, with high efficiency, by incorporating certain osmotic regulators like sorbitol or mannitol into the medium. Salt tolerant cell clones were obtained from calli grown on sodium chloride added media, which have been regenerated into plantlets; drought tolerant clones were obtained from calli subjected to PEG. Both the salt tolerant and drought resistant plants are now grown under field conditions and are being further evaluated with seawater. Studies have also been conducted to understand the basic regulatory mechanisms associated with growth and differentiation; specific proteins and certain isozymes were found to be present preceding organogenesis.

In maize, callus cultures have been employed for the study of anthocyanin biosynthesis *in vitro* as well as for plant regeneration. Somatic embryogenesis has been observed in root and glume cultures. Anthocyanin synthesis has been found in endosperm cultures and root cultures. Characterization of the synthesized compounds indicated that they were similar to those found in intact plant. Cross-feeding studies with the use of certain precursors and intermediates like cinnamic acid, caffeic acid and coumaric acid to competent root callus of CI resulted in pigment synthesis, suggesting that CI, an allele of C1, acts before the <sup>15</sup>C

intermediate in the known biosynthetic pathway.

### Induction of *In Vitro* Flowering

In groundnut, cotyledons with and without embryo axes cultured on Blaydes media supplemented with cytokinins produced flower buds and flowers within three weeks of culturing. Pod formation from gynophores of the flowers was also achieved in a chemically defined medium with hormones under dark conditions. In sunflower, cotyledons developed shoots in 6-8 days and then these shoots developed flowers with high frequency, which bloomed in 60-70 days on the medium used for induction. In safflower, shoot and capitula initiation was observed from the cut end of the inner surface of the cotyledons, 20-40 days after inoculation. Embryo development was observed and six seeds were harvested. This phenomenon of direct flowering offers a unique system in the study of the physiological and molecular basis of flowering and in the study of the physiological and molecular basis of flowering and in the transition of mitosis to meiosis under defined conditions.

In fruit crops like mango, guava and grape, callus has been induced from cotyledons, embryo, mesocarp and leaf. Phenolic absorbents like activated charcoal, ascorbic acid, polyvinyl pyrrolidone (PVP) have been used in the medium. Studies on morphogenesis indicated root formation in mango cultures. Callus initiation was accomplished in mango from cotyledon, mesocarp, embryo and leaf. Studies on morphogenesis and plantlet regeneration is still a major problem; however, root formation was observed.



## Future Directions in Plant Genetics Research

The major lines of work proposed to be undertaken include: Search for non-allelic genes in rice; incorporation and study of scentedness in local rice germplasm; characterization of enzymes in anthocyanin biosynthesis to understand gene regulation in maize; exploitation of somaclonal variation for improvement of certain economically important higher plants; establishment of cell suspension cultures for the selection of desirable characters like protein quality, disease resistance, salt tolerance, etc.; improving the efficiency of haploid from glumes and roots of haploid genotypes; *in vitro* studies on the behaviour and expression of transposable elements like a-Dt, Ac-Ds, etc. in maize; protoplast isolation, culture, regeneration and fusion studies with wild and cultivated species in order to transfer certain desirable characters like disease resistance; and DNA uptake (micro injection) studies utilizing anthocyanin genetic markers of maize.

## Selected Publications

1. Reddy G M, Genetic control of leucoanthocyanidin formation in maize, *Genetics*, **50** (1964) 485-89.
2. Reddy G M, Genetic basis of protein quality in maize, in *Symp Accl Genet Imp Ind Pl Resour*, IARI, New Delhi, *Indian J Genet Pl Breed* (Sp Vol), **24**(4) (1965) 27.
3. Reddy G M, Analysis of gene action sequence in anthocyanin formation in maize, *Proc First All India Congress of Cytology and Genetics*, *J Cytol Genet Cong* (Suppl I) (1971) 321-25.
4. Reddy G M, Role of induced morphological, grassy and dwarf mutants in evolving new plant types in *Oryza sativa* L, *Proc Symp on Recent trends and contacts between Cytogenetics, Embryology and Morphology*, Nagpur, 1976, 1-9.
5. Reddy G M, Induced dwarfs and semi-dwarfs in rice breeding, *Proc Symp on Improvement of rice production through nuclear techniques*, FAO/IAEA, Jakarta, Indonesia, 12-14 October 1977.
6. Reddy G M, Gene action in anthocyanin biosynthesis in maize, *J Cytol Genet*, **15** (1980) 116-22.
7. Reddy G M, Induced grain type and dwarf mutants in rice breeding, *Proc Second All India Cong Cytol & Genet*, Udaipur, 20-24 October 1978, 143-45.
8. Reddy G M, Role of induced short culmed mutations in rice improvement, *Proc Dunn and Dobzhansky Memorial Symposium on Genetics*, Mysore, 1976, 259-64.
9. Reddy G M, Induced non-allelic and other dwarfs in local cultivars in rice breeding, *Proc Third Int Cong*, Vol 1, Sect 6, SABRAO, Australia, 13-16 February 1977, 10-12.
10. Reddy G M & Coe E H, Inter-tissue complementation. A simple technique for direct analysis of gene action sequence, *Science*, **138** (1962) 149-50.
11. Reddy G M, Tissue culture and mutational studies in rice improvement, *Proc Symposium on Tissue Culture of Economically Important Crop Plants*, National University of Singapore, April 1981, 7-10.
12. Reddy G M, Callus initiation and plant regeneration from haploid internodes in rice, *Proc International Symposium on Plant Cell and Tissue Culture in Crop Improvement*, Calcutta, December 1981, 113-18.
13. Reddy G M, Recent trends in plant genetics research, in *Recent trends in botanical researches*, edited by R P Sinha (R P Roy Commemoration Fund, Patna) 1984, 82-91.
14. *Proceedings, Int Symp on Gene Structure and Function in Higher Plants*, edited by G M Reddy and E H Coe (Jr) (Oxford & IBH Publ, New Delhi) 1985.
15. Reddy G M, Anthocyanin biosynthesis in maize—A model system in the study of gene action and gene regulation, in *Gene Structure & Function in Higher Plants*, edited by G M Reddy & E H Coe (Jr) (Oxford & IBH Publ, New Delhi) 1985, 81-89.

## V V Modi

The importance of rhizobia in biological nitrogen fixation is well established. We have been working on the genetics and physiological aspects of rhizobia for nearly two decades. We convincingly demonstrated the genetic transformation of DNA in *Rhizobium*. We also showed interspecific and intraspecific transformation in this system. The genetic transformation system was further characterized and the requirement of the competence factor for transformation was shown. Conditions favouring the production of competence factor and hence better transformation frequency were established. Different *Rhizobium* species were mapped using transformation for antibiotic resistance, auxotrophy, gelatinase production and many other markers.

While working on transformation in one strain of *R. japonicum*, a lysogenic phage was induced. This phage was subsequently isolated and its structure and properties were characterized. The phage was mapped for many markers. Adsorption of the phage to the host was characterized; the receptor site was shown to contain a few sugars, and was blocked by the exopolysaccharide produced by the organism. An enzyme exopolysaccharide depolymerase was found in the phage lysate.

Biochemical and physiological characterization of *Rhizobium* and nodules was also done. Production of heme and enzymes involved in its metabolism was studied. Enzymes involved in the metabolism of the storage product, polyhydroxybutyrate granules, were characterized. It has been suggested that PHB may have a role as the energy reserve for the organism. Energy and reducing power are important requirements for nitrogen fixation. Nodule studies suggested that IDH may be supplying the reducing power for this process. The presence of ATPase was established in bacteria, bacteroid and nodule cytosol.

Enzymes of ammonia assimilation were characterized in bacteria and nodule. GS was found to be the major enzyme involved in ammonia assimilation. Asparagin synthetase was suggested to have an important role in ammonia assimilation in soybean. The effect of a GS inhibitor, MSX, on ammonia assimilation was studied; it was shown that the organisms adapt themselves to this inhibitor and start using GDH as an alternative pathway for ammonia assimilation<sup>1-5</sup>. We have also reported the isolation of catechol like siderophore from cowpea *Rhizobium* RA-1 which may be responsible for Fe<sup>2+</sup> transport.



### Microbial Production of $\beta$ -Carotene (Carotenogenesis in *Blakeslea trispora*)

Mating of single strains of the heterothallic mould *Blakeslea trispora* results in increased carotenogenesis; this has been attributed to the production of a sex hormone, trisporic acid (TA). Modi and his team have observed that increased carotenogenesis (1) stimulates the activity of a membrane-bound protease, which probably inactivates a proteinic inhibitor of the carotene biosynthetic pathway, (2) increases cyclic AMP levels, thereby circumventing the adverse effect of catabolite repression on carotenogenesis, and (3) stimulates the activity of the enzymes involved in carotene synthesis (MVAKinase), and in the generation of ATP and NADPH. Ultrastructural studies of single and mated cultures reveal that TA brings about thickening of the cell wall of mated cultures and increases their lipid content.

Fermentation studies have revealed that *B. trispora* can be exploited for the simultaneous production of  $\beta$ -carotene and  $\beta$ -glucosidase. High concentrations of phosphate enhance carotene production markedly, thereby increasing the potentiality of this mould to produce  $\beta$ -carotene on a large scale<sup>6-9</sup>.

### Microbiology and Environmental Problems

A strain of *Rhodotorula rubra* was found to be useful in the treatment of petrochemical oil sludge. As a result of the treatment, BOD, COD, oil content and phytotoxicity of the oil sludge decreased. *Rhodotorula rubra*, which showed the capacity to degrade a wide variety of hydrocarbons, was found to harbour a plasmid like DNA.

Chloroaromatic compounds are highly toxic and refractile to biological attack because of the presence of chloride atom on the aromatic ring. Conditions were standardized to obtain effective dechlorination, using immobilized *Aspergillus niger* mycelium. The above-mentioned systems have the potential for use in the treatment of environmental pollutants<sup>10-12</sup>.

### Avoidance of Microbial Spoilage during Fruit Ripening

It was demonstrated that ripening of mangoes occurs through inactivation of proteinic inhibitors present in unripe mangoes. The results from this laboratory provided proof that mangoes produce ethylene. *In vitro* studies suggested that ethylene may induce ripening in mango by inactivating the proteinic inhibitors.

Of the other hormones, abscisic acid has been shown to enhance the ripening of mangoes, whereas gibberellic acid, indole acetic acid and kinetin delayed the ripening process. The effect of ABA could involve its action at transcriptional and/or translational level.

In respect of storage of fruits at low temperature, studies on the development of chilling injury in low temperature stored mangoes were carried out. Chilling injury of mangoes was found to result in accumulation of minerals which affect the metabolic activity of the injured tissue. The enzyme invertase with two pH optima, one at 37°C and the other at 0°C, has been shown to be present in mangoes. Low temperature storage has been shown to have its effect at the level of mitochondria, cell membrane and membrane-bound ATPase.

The intermediates of carotenoid biosynthetic pathway during the ripening of mangoes were identified to be regulated by phosphatases<sup>13-15</sup>.

### Selected Publications

1. Nautiyal C S & Modi V V, Regulation of glutamine synthetase I from *R. meliloti*, *Curr Microbiol*, **5** (1981) 329.
2. Shah K S, desouza S and Modi V V, Studies on transducing phage M-1 for *R. japonicum* D211, *Arch Microbiol*, **130** (1981) 262.
3. Shah K S, Patel C & Modi V V, Linkage mapping of rhizobium Japonicum D211 by phase M.1 mediated transduction, *Can J Microbiol*, **29** (1983) 33.
4. Apte R & Modi V V, A receptor from a host plant....binds to Rhizobium meliloti strains, *Experientia*, **39** (1983) 509.
5. Modi Mayuranki, Shah K S & Modi V V, Isolation and characterization of catechol-like siderophore from cowpea Rhizobium RA-1, *Arch Microbiol*, **141** (1985) 156.
6. Dandekar S & Modi V V, Involvement of CAMP in carotenogenesis and cell differentiation in *Blakeslea trispora*, *Biochim biophys Acta*, **628** (1980) 398-416.
7. Govind N S, Mehta Bina, Sharma Minakshi & Modi V V, Protease and carotenogenesis in *B. trispora*, *Phytochemistry*, **20** (1981) 2483.
8. Dholakia J N & Modi V V, Fermentative production of  $\beta$ -carotene and extracellular  $\beta$ -glucosidase by *Blakeslea trispora* grown on cellobiose, *Eur J appl Microbiol Biotechnol*, **15** (1982) 3335.
9. Dholakia J N & Modi V V, Regulation of carotenogenesis by inorganic phosphate in *B. trispora*, *J gen Microbiol*, **130** (1984) 2043.
10. Shailubhai K, Rao N N & Modi V V, Microbial treatment of petroleum-industry sludge and its evaluation, *Management of environment*, edited by B Patel (Wiley Eastern Ltd., New Delhi) 1980, 170-80.
11. Shailubhai K, Sahasrabudhe S R, Vora K A & Modi V V, Degradation of-chlorobenzoates by *A. niger*, *Experientia*, **40** (1984) 406.
12. Shailubhai K, Rao N N & Modi V V, Treatment of petroleum industry oil sludge by *Rhodotorula* sp, *Eur J appl Microbiol Biotechnol*, **19** (1984) 437.
13. Majumdar Gopa & Modi V V, Effect of plant hormones and temperature on membrane permeability of mango fruit, *Indian J exp Biol*, **18** (1980) 325.
14. Majumdar, Gopa, Modi V V & Palejwala V A, Effect of plant growth regulator on mango ripening, *Indian J exp Biol*, **19** (1981) 885.
15. Toraskar Manjiri V, Palejwala V A & Modi V V, Peroxidase and chilling injury in banana fruit, *J Agric Fd Chem*, **32** (1984) 1352.



## S K Mukherjee

The polyphasic and polybasic nature of soil clay colloids in their hydrogen form in aqueous suspension has been studied in a variety of ways. Their electrochemical behaviour has shown many interesting facets, unlike systems in true aqueous solution. Chemically, the clay colloids are basically crystalline aluminosilicates. The surface of the colloidal clay particles is capable of exchanging with cations. In the hydrogen form, the system is acidic and is amenable to potentiometric and conductometric titrations. These studies have given a clear understanding of what constitutes soil acidity and the role played by cations like  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$  and  $\text{Mg}^{2+}$  in amending acidity, as well as of the binding characteristic of these cations. A follow up of this work demonstrated that the characteristic features of the electrometric titration curves could be used to identify the nature of clay minerals in soil clay. Clay is the finest fraction of soil particles in association with silt, sand and humus. It dominates the soil properties of which cation exchange is the most important and interesting. The results of study of the cation exchange characteristics of clays have been reported in a number of papers. The exchange equilibrium has been studied in a variety of ways, namely, by varying the valency, nature and concentration of cations using different silicate minerals.

These investigations have revealed many interesting facets of exchange reactions. They are basic to the understanding of the physicochemical behaviour of soils as a substrate for nutrient supply to growing crops. The exchange behaviour of homoionic and heteroionic clays has been studied by means of their exchange isotherms which reveal not only some surface characteristics of the clays, but also differences in the behaviour of various ionic interactions, namely, selectivity coefficient, ionic antagonism, preferential ion adsorption and nonequivalent binding sites on clay surface. These studies have been wide-ranging, covering almost all the clays that are ordinarily present in soil, and numerous cations, including the macro- and micro-nutrients.

The exchange equilibrium studies have been extended to synthetic exchange substances, such as the cation and anion exchange resins and heteropoly salts using anions and complex ions like those of coordination complexes, quaternary ammonium and dye ions.

An interesting development resulting from ion exchange studies of clays is the clay membrane electrodes, which could be used to measure the activities of cations in  $10^{-3} - 10^{-5}$  molal concentration range not only in pure solutions but also in colloidal

suspensions of clays. This technique has been extended to synthetic resin membrane electrodes and to electrodes prepared in a variety of ways, in order to obtain selective electrodes. The membrane electrodes have been used successfully to understand many of the finer features of exchange equilibria, in addition to supplementing information obtained on such equilibria through chemical analytical procedures. Because of the possibility of measuring the activities of the interacting ions, it has been possible to determine more accurately the equilibrium constants of exchange reactions. From the data at various temperatures, the thermodynamic properties of the exchange systems could be evaluated.

Humus, the microbial decomposition end product of organic matter in soil, is an

important soil component, even though it constitutes no more than 1% of our soils. Its importance lies in the manner in which even in this small proportion it influences the physicochemical properties of soil. Humus can be fractionated into humic, fulvic and hymatomelanic acids, of which the first two are more effective than the third one. Chemically, these are lignin/cellulose-protein polymeric compounds containing nitrogen as a probable constituent of heterocyclic compounds. They are very difficult to fully characterize chemically, but are amenable to physicochemical measurements. The latter include electrochemical titrations, viscosity measurements and fluorescence excitation spectroscopic measurements. The polyelectrolytic nature of the component acids of humus could be established through electrometric and viscometric studies.



## B R Murty

Murty has to his credit outstanding contributions in genetics, including radiation genetics and cytogenetics, plant breeding, statistics and evolution made during the 35 years of his research career. Murty's researches involve a unique combination of genetics, statistics and cytogenetics with productivity and creation of new schools of thought in different fields such as evolutionary divergence of biological populations by distance analysis, canonical and factor analysis, selection methodology, mutation breeding, cytogenetic regulation of recombination, use of nuclear tools for detecting and overcoming crossability barriers, divergence in species, and morphogenesis and interdisciplinary research in genetics to elevate crop production in cereals, legumes and oilseeds under semi-arid tropical conditions making use of the advances made in the field of atomic energy.

Murty joined the Indian Agricultural Research Institute and started in 1961 the School of Biometrical Genetics, which laid the foundations for the modification of the earlier concepts in plant breeding. Murty was a Senior Associate with the US Atomic Energy Commission at the Brookhaven National Laboratory. The School of Biometrical Genetics created by him is considered to be one of the strongest and most productive in the world. Its impact has modified the current

breeding procedures and is felt in all the crop improvement programmes in India; it has also demonstrated the success of interdisciplinary approach to selection.

His major contributions in fundamental genetics have been in the field of multivariate analysis in the assessment of genetic divergence in biological populations, changes in components of variance in relation to breeding systems, genetics of adaptation in food crops, disruptive selection for crop improvement and the genetic regulation of recombination in wheat, *Pennisetum* and *Brassica*, use of mixed pollen to preserve diversity, mutation breeding for disease resistance, and components of association, and theoretical studies for improving the efficiency of designs for estimating genetic parameters in plant breeding using fractional diallel and triangular designs, which have cut down the crossing programme substantially with improvement in efficiency. He established for the first time a relationship between the changes in the regulation of recombination with the genetic load and fitness in crops with diverse breeding systems and put forth a new theory which has replaced the earlier concepts on the changes in chiasma frequency under inbreeding and has now been accepted internationally. His work on disruptive selection has unequivocally resolved an international controversy

about its role in creating divergence and release of variability by a unique combination of statistical assessment of the divergence and components of variance analysis and as a result it has been possible to apply it to crop improvement; as a result of this work two varieties of mustard were released. He has also shown that genetic load, as a major factor, is related to fitness and has a regulatory role in chiasma frequency and release of variability. His basic work, with emphasis on selection for developmental traits, demonstrated in both self- and cross-pollinated crops, has been accepted by the International Atomic Energy Agency and has helped in the simultaneous improvement of yield, grain quality and disease resistance of plants and has modified the concepts on the stage of selection and the relative importance of selection for specific attributes.

A new approach to the study of evolutionary trends in biological populations has been established through the use of improved biometrical criteria. He created a new school of thought utilizing multivariate analysis, including factor analysis, for the assessment of genetic divergence in crops with different breeding systems.

This assessment was carried to the logical conclusion by evolving better varieties in cereals and oilseeds for establishing a one to one correspondence of the estimates of divergence with the components of genetic variation and the advance under selection. His work on cataloguing and classification of genetic stocks of world collections and the information retrieval system has been used as a model for all crops by FAO, the

International Atomic Energy Agency and several other international institutions.

As coordinator of the All India Millet Improvement Project, Murty was responsible for the release of four hybrids of pearl millet, three new male steriles, three varieties of *ragi* and two varieties of *Setaria* for all-India cultivation. The development of downy mildew resistant male steriles of pearl millet and their hybrids is considered unique in mutation breeding.

At international level, he was a Member of Scientific Panels in Breeding for Disease Resistance, World Gene Pools and Biology of Adaptation and is serving as the International Coordinator for the Biology of Adaptation of Grain Legumes. He was the Chairman of the UM Section of IBP and Secretary of the SCIBP in India. As Director of FAO Training Programme, he conducted a multi-disciplinary training programme on crop improvement with relevance to countries of Africa, Asia and Latin America in wide ranging environments in the production of maize, sorghum and millets. His studies on tropical adaptation have thrown light on its complexity at biochemical and physiological levels. As a Member of the National Animal Breeding Panel and the Cattle Breeding and Crop Panels of the National Commission on Agriculture, he was involved in the formulation of research programme, including coordinated projects relating to cattle, sheep, goats and poultry. As a National Panel Member on Agriculture Economics, Statistics and Marketing and as a Panel Member for Fertilizers and Chemicals of Indian Standards Institution, he is conversant with the problems of these allied disciplines directly influencing agricultural



production and has been providing the necessary direction to the programme.

Murty was elected Fellow of the Indian National Science Academy in 1973, Fellow of Royal Statistical Society, London, in 1974, and Fellow of Indian Academy of Sciences in 1974. He is a Member of the Editorial Board of *Theoretical and Applied Genetics* (Germany) and *Genetica Agraria* (Italy).

Murty has made major contributions in the field of genetic statistics through the use of sophisticated mathematics and computer techniques in the genetic analysis of wheat. He was a member of an international panel of scientists who reviewed the present breeding procedures to modify them to meet the world food needs for the 21st century, and prepared a comprehensive document, which was published in 1979 as a reference book "*Plant Breeding Perspectives*" (Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands).

His other contributions in biometrical genetics are: (a) Optimal fractional diallel using circulant and triangular designs, and study of their relative efficiencies in the estimation of genetic parameters; (b) partitioning components of association into linkage, pleiotropy and physiological association; (c) use of convolutions in the estimates of genetic parameters; (d) computer programmes for the above analysis and information retrieval of world germplasm data on sorghum and millets; (e) establishing the role of disruptive selection in *Brassica* improvement; and (f) study of the role of maternal effects in disease resistance, yield and adaptation in some cereals and oilseeds.

## Radiation Genetics

His studies in radiation genetics include: (a) evolving downy mildew resistant male-steriles and hybrids in millet; (b) production of new plant types in sorghum, cowpea and mung bean and sesamum; (c) basic studies on intra- and inter-specific differentiation; (d) improvement of nutritional quality and yield in barley, breaking the adverse association between yield, protein content and seed size; and (e) isolation of non-nodulating mutant in cowpea useful for basic studies on dinitrogen fixation in legumes.

## Recent Work (1975-85)

Murty's work during the past 10 years, based on the adoption of interdisciplinary approach to selection involving genetics, biochemistry, soil science and physiology, etc. for improved and stable crop productivity in dryland agriculture, has helped in the isolation of genotypes in different crops with better root activity, efficient nutrient uptake and transfer and sustained activity; some enzymes enabling adaptation under moisture stress, in millet, barley, wheat, chickpea and *Brassica*, have been identified using nuclear and allied techniques. Recently, the nature of cytoplasmic differentiation in millet was brought out by him using multivariate analysis of characters involving biochemical and ultra-structural differences in cell-organelles and developmental traits using near-isogenic lines.

As the Chief of Nuclear Research Laboratory, New Delhi, he catalysed interdisciplinary research involving the use of nuclear tools and techniques for the solution of problems of semi-arid agriculture in the tropics.

During the past 5 years, Murty has been a United Nations expert in Venezuela. Through mutation breeding work using interdisciplinary approach, he has evolved eight lines each in sorghum and cowpea, four in mungbean and five in sesamum with substantial (80-110%) increase in yield over the best available elite genotypes, including hybrids.

In 1984, Murty examined the present status of multivariate analysis in real world situations, to identify gaps between the user needs and the available statistical theory and tools in multivariate analysis as applied to biology and social sciences. As a result he indicated future areas of research into the methodology to overcome the deficiencies identified, with special reference to "missing observations in multivariate analysis" and analysis of data from complex designs in intercropping experiments in agriculture.

### Selected Publications

1. Kadam B S & Murty B R, A new linkage in tobacco, *Nature, Lond*, **171** (1953) 1028-29.
2. Murty B R & Swaminathan M S, Cytogenetic studies in derivatives of *Nicotiana rustica* x *N. tabacum*, *Euphytica*, **6** (1959) 227-36.
3. Swaminathan M S & Murty B R, Aspects of asynapsis in plants. I. Random and non-random chromosome associations, *Genetics*, **44** (1959) 1271-80.
4. Murty B R, Murty G S & Pavate M V, Studies in quantitative inheritance in *Nicotiana tabacum*, *Der Zuchter*, **32** (1962) 361-69.
5. Murty B R, Mathur J B L & Arunachalam V, Self-incompatibility and genetic divergence in *Brassica campestris* var Brown sarson, *Sankhya (Ser B)*, **27** (1965) 271-78.
6. Murty B R, Arunachalam V & Anand I J, Diallel and partial diallel analysis of some yield factors in *Linum usitatissimum*, *Heredity*, **22** (1967) 35-41.
7. Murty B R, Selection limits. Contributions in Statistics and Agricultural Sciences, *J Indian agric Stat (Sp Vol)*, **20A** (1968) 295-305.
8. Chandrasekhariah S R, Murty B R & Arunachalam V, Multivariate analysis of genetic divergence in Ew-sorghums, *Proc natn Inst Sci India*, **35B** (1969) 172-95.
9. Roy N N & Murty B R, A selection procedure in wheat for stress environment, *Euphytica*, **19** (1970) 509-21.
10. Murty B R & Arunachalam V, The nature of genetic divergence in relation to breeding system in crop plants, *Indian J Genet*, **26A** (1966) 188-98.
11. Murty B R, Arunachalam V & Jain O P, Factor analysis in relation to breeding system, *Genetica*, **41** (1970) 1-11.
12. Murty B R, Arunachalam V, Doloi P C & Ram J, Effect of disruptive selection for flowering time in *Brassica campestris* var brown sarson, *Heredity*, **28** (1972) 287-95.
13. Murty B R, Biometrical classification of the genus Sorghum, in *Sorghum in the seventies*, edited by L R House and Rao (IBH Publishing, New Delhi) 1973, 14-38.
14. Ranga Rao V & Murty B R, Fractional diallel analysis of regulation of recombination in *Triticum aestivum*, *Cytologia*, **37** (1973) 83-93.
15. Singh B B & Murty B R, A comparative analysis of biparental mating and selfing in pearl millet, *Theoret appl Genet*, **43** (1973) 18-22.
16. Murty B R, Role of developmental traits in breeding for disease resistance in some cereals, *IAEA Panel Symp Mutation Breeding for disease resistance*, Vienna, PL 412, **10** (1977) 93-105.
17. Murty B R, Biometrical genetics in agriculture, in *Symposium on Basic Sciences and Agriculture*, New Delhi, 11-13 October 1975 (Indian National Science Academy, New Delhi) 1977, 25-33.
18. Rana B S & Murty B R, Heterosis and components of genetic variation for protein and lysine content in some grain sorghums, *Theoret appl Genet*, **45** (1975) 225-30.
19. Devaratnam A A, Arunachalam V & Murty B R, Quantitative evaluation of inter-varietal hybrids of *Brassica campestris*, *Theoret appl Genet*, **48** (1976) 1-8.
20. Verma M M, Chahal G S & Murty B R, Limitations of conventional regression analysis—A proposed modification, *Theoret appl Genet*, **53** (1978) 89-91.
21. Ram J & Murty B R, Changes in genetic background under selfing influencing selection and the expression of self-incompatibility in *Brassica campestris* var brown sarson, *Genetica*, **51** (1979) 45-53.



22. Govil J N, Mukherjee B K & Murty B R, Studies on nature and magnitude of genetic parameters estimated in the original and advanced generations of certain sorghum hybrids, *Z Pflanzen*, **82** (1979) 340-48.
23. Murty B R, Breakthrough in breeding for resistance to downy mildew in pearl millet, *EPPO Bull*, **10** (1980) 311-15.
24. Murty B R, Bhakta S T, Thakur S R, Mehta S L & Nam Prakash, Mutation breeding for downy mildew resistance in pearl millet-nucleo-cytoplasmic interactions in disease resistant lines, in *Induced mutations for disease resistance in crop plants*, (FACA publ S TJ/1633, Vienna) 1983, 119-24.
25. Murty B R, Tobardo F & Reisono A, Interdisciplinary approach to selection in local sorghums for adaptation and disease resistance, *IAEA Tech Doc No. 305* (1984) 141-72.
26. Valera M & Murty B R, Genetic and physiological relationships in N, P and K mobilization in some sorghum mutants and parents, *Theoret appl Genet*, **69**, 353-59.
27. Murty B R & Federei W T, Missing observations in multivariate analysis, *Technical Publ No. 858-M, Biometrics Unit, Cornell University, Ithaca NY*, 1985, 1-21.

## B P Pal

Pal's main research has been in the field of genetics and plant breeding and the crop on which he has worked most is wheat. For his PhD degree at Cambridge, he worked on the problem of hybrid vigour in wheat; he continued his keen interest in the crop in later years. Soon after he joined at Pusa, he was entrusted with the wheat breeding work. At that time the position was that resulting from the work of Sir Albert Howard and others; varieties were available which were reasonably high yielding and had good grain quality, but the problem of diseases had hardly been tackled. Pal took up the question of breeding for disease resistance in earnest and through his own work and that of his associates, he was able to release a series of wheats known as the 700 series and the 800 series, which were characterized by not only good yield and grain quality but also a considerable degree of resistance to the three rusts of wheat which do a great deal of damage to the crop in India. These wheats have occupied in the past, it is estimated, several million acres and the value of the work was worth crores of rupees to the farmers. Perhaps Pal's most outstanding achievement in wheat breeding was the evolution of the variety NP 809 as a result of 18 years' work combining resistance to the different races of the three rusts, available in foreign varieties, with the desired agronomic and grain qualities of the best Indian wheats.

NP 809 is considered to be a landmark in the history of wheat breeding and attracted the attention of Henry Wallace, formerly Secretary for Agriculture, USA, who in an article wrote as follows:

"Hybridization has been used by the Indian Agricultural Research Institute to produce a strain of wheat, New Pusa 809, which is resistant to all three types of the damaging disease known as wheat rust. The rust can attack in a red, yellow or black form, and in the past has taken a heavy toll of the country's wheat crop. Though for a long time it has been possible to breed wheat resistant to one form, this is the first strain that will combat all three. A high-yielding and hardy variety, New Pusa 809, may well be the answer to a serious menace."

Pal was also successful in incorporating into his wheats a high degree of resistance or even immunity to another very serious disease of wheat, viz. loose smut. This was a great boon, because it rendered unnecessary the tedious hot water treatment which otherwise had to be applied to wheat seeds to free them from this seed-borne disease. When he gave up wheat breeding some years ago to his successors, he left behind a great deal of valuable material. He had built up very comprehensive collections of Indian and foreign wheats and also hybrid materials of great value.

---

Formerly, Director-General, Indian Council of Agricultural Research & Chairman, National Committee for Environmental Planning and Co-ordination, New Delhi; Residence : P-11, Hauz Khas Enclave, New Delhi-110016.



### Potatoes

Pal laid the foundations of potato breeding work. Along with his associate, Dr. Pushkarnath, he initiated a strong programme for potato research. He was in charge of the Potato Breeding Sub-station at Simla and the work done there laid the foundations for the Central Potato Research Institute which came later.

### Tobacco

Pal also did some work on tobacco. He was the first to make a serious study of the tobacco leaf curl disease in India and to show that it was caused by a virus. He was in charge of the Virginia Tobacco Sub-station at Guntur which did some useful work in the early years before the Central Tobacco Research Institute was founded.

### Plant Introduction

Although introduction of economic plants into India has a long history, systematic work on modern lines was begun under a scheme financed by the ICAR of which Pal was incharge. His

associate, Dr H B Singh, went on to develop this work on a big scale and plant materials of great potential value have been introduced into the country. The organization has been expanded and has taken the shape of the National Bureau of Plant Genetic Resources of ICAR.

### Other Research

Pal also found time to do work on a number of other items, including hybrid vigour in a number of economic plants and also has paid a great deal of attention to ornamental horticulture, which has been one of his principal side activities in research. He has bred many varieties of roses, including 'Dr Homi Bhabha', 'Raja of Nalagarh', 'Mechak' and 'Poornima' among the Hybrid Teas, and 'Delhi Princess', 'Banjaran', 'Kumkum' and 'Suryakiran' among the Floribundas, which have been well received. He has also raised Bougainvillea varieties, of which 'Dr R R Pal' is the most popular. He is known not only in India but abroad as a rosarian and his book, *The Rose in India*, has been well received.

## R S Paroda

### (1) New Varieties Evolved

As a consequence of the work of Paroda and his colleagues, the following varieties have been evolved and released :

<i>Crop</i>	<i>Varieties</i>	<i>Year of release</i>	<i>Centre/State</i>
Sorghum	1. S.S.G. 59-3 (Multicut)	1975	Centre
	2. Haryana Chari (Singlecut)	1975	Centre
	3. HC 136 (Singlecut)	1981	Centre
Oat	1. Haryana Javi (Multicut) (HFO 114)	1974	State
	2. OS-6 (Singlecut)	1981	Centre
	3. OS-7 (Singlecut)	1981	State
Cowpea	HFC 42-1 (Singlecut)	1975	State
Guar	1. HG 75 (Seed)	1981	Centre
	2. HFG 119 (Fodder)	1981	Centre
	3. HFC 182 of Clusterbean for grain and gum	1984	State
Gram	H 75-35	1984	State
Arhar	H 77-216	1984	State
Barley	BH 75	1984	State

### (2) Basic Research Contributions

A model for studying the synchrony of ear emergence and other ear attributes was proposed for the first time in barley<sup>1</sup>.

Genetic architectures for yield and related attributes were studied in different environments in wheat and barley. The sensitivity and stability of additive and dominance components of genetic variance were studied systematically for the first time and it was found that the

former was a more stable component of the two<sup>2</sup>.

It was observed that array means could serve as good indicators of the general combining ability of the parents. Similarly,  $F_2$  generation could also be used to study the gca effects only and not the sca effects. Further, it was established that the analysis using models, excluding parents, provided better estimates of combining ability variances<sup>3</sup>.

---

Director, National Bureau of Plant Genetic Resources, New Delhi-110012; Residence : B-37, Krishi Vihar, Joseph Broz Tito Marg (opp. Panch Sheel Enclave), New Delhi.



The genetic architecture of different forage yield and quality attributes has been worked out in crops like sorghum, bajra and oats. The information obtained provides a basis for further genetic improvement in these crops. Additive genetic variance is present in a sizeable proportion. Low tannin was found to be dominant over high tannin content and thus breeding for lines relatively free from tannin is possible<sup>4</sup>. Similarly, genetics of HCN, *in vitro* dry matter digestibility and protein content were studied.

Extensive studies on inter-specific hybridization in *Eu-Sorghums*, oats, cowpea and guar have revealed that species crosses can throw the desired genetic variability for most of the forage attributes; heterosis recorded in some of the crosses was spectacular (2-3 fold over best check).

Cytogenetic studies have revealed that the species of *Eu-Sorghums* differ significantly in their nuclear DNA, mitotic index and heterochromatin<sup>5</sup>. DNA differences were found to be due to variation in heterochromatin content, although the species did not differ in their total chromosome length measured at pachytene. This information has, therefore, opened up new avenues.

Reciprocal differences were reported for the first time in maize  $\times$  teosinte hybrids for different forage characters<sup>6</sup>. Further intensive experimentation has revealed the presence of apomixis (facultative type) in teosinte for the first time and it was observed that the embryo development was through nucellar cells.

Preliminary investigations on the biochemical basis of resistance to red leaf spot diseases in forage sorghum have

revealed that resistant sources such as *S. roxburghii* and PJ7R are low in tannin content. Further studies on phenolic compounds are in progress and these are likely to provide useful information for disease resistant breeding.

Correlation studies on mean performance and both the parameters of stability (*b* and *S-2d*) have revealed that all three estimates are independent. However, convincing evidence regarding different gene systems controlling both the linear and non-linear components of  $G \times E$  interactions was provided by Dangi and Paroda<sup>7</sup>. Two different parents showed dominance for the two parameters of stability separately, as evident from their array means. This, therefore, has specific significance, because parents showing dominance for linear response (*b*) or parameter of stability (*S-2d*) could be usefully exploited in future breeding programmes aimed at breeding of high yielding stable forage varieties.

Paroda *et al.*<sup>8</sup> and Karwasra *et al.*<sup>9</sup> have observed that a fewer rather than a complete set of genotypes evaluated earlier could also be used successfully in predicting the mean performance, especially of the stable genotypes. Also, the use of as low as one stable genotype at a time could be made to predict the yields of the remaining stable genotypes. However, the use of only one genotype for the purpose of prediction appeared to have its own limitations, particularly when the unstable genotype is taken into account. Prediction appeared to be feasible particularly for stable genotypes in both the methods used. It has been reported that the stable genotypes could also be used to assess the relative response patterns of the various

genotypes tested earlier in a few contrasting environments. Thus, information regarding the practical implication of the genotype-environment interactions is now becoming available and could be used successfully in a breeding programme aimed at developing high yielding stable genotypes.

### (3) Applied Research Contributions

Studies on phenotypic stability have indicated the need for breeding stable varieties, as most of the varieties released in the past (before 1971) are invariably unstable over environments in their yield performance. Newly released/pre-released varieties like Haryana Javi-114 of oats, HFC 42-1 of cowpea, HFG 75 of guar, HFM 39 of metha, SSG 59-3 of Sudan grass and S 136 of sorghum are superior in their stability in addition to being high yielding and nutritious. Methods have been devised by which prediction of genotypes across environments as well as across genotypes is possible<sup>10</sup>. This indeed is a major contribution in the field of breeding for increased stability.

Based on correlation of path-coefficient analyses in different crops, it has been possible to work out morphological parameters which could be used in selection programmes for simultaneous improvement in forage yield and quality. Leaf weight per plant has been found to make maximum contribution to dry matter accumulation; its positive association with protein and IVDMD has further revealed its importance in forage breeding programme. Leafiness has also been found to be correlated with intake and thus breeding in forages must aim at the improvement of this attribute. For most of the forage crops, plant height, number of tillers and leaf area per plant have been

found to be the most important characters. It has also been established that improvement in leaf characters would result in increased stability of dry matter production.

By using mating designs like diallel and line  $\times$  tester, it was possible to identify the best combining parents for various yield and quality attributes in almost all important forage crops. These parents have been used extensively and best crosses have been exploited further through North Carolina mating designs. Undesirable linkages have been broken and very useful transgressive segregants possessing leafiness, high yield and quality and resistance to diseases, etc. have been selected in advanced generations for further intensive testing.

Extensive studies on forage sorghum hybrids have revealed the presence of heterosis up to the extent of 120% of green fodder yield and up to 160% for dry matter production over best check. Most stable hybrids have been identified and these have shown least depression from  $F_1$  to  $F_2$ . These hybrids are much superior to check in poor environments. Male sterile lines like GIA and 10626A are good combiners having low tannin content and thus should be exploited.

A new crossing technique proposed for guar<sup>12</sup> has proved to be quite simple as well as efficient by almost 2-3 times over the technique in use. A new efficient crossing technique for oats has been developed<sup>13</sup>.

Screening of forage sorghum genetic stock for hydrocyanic (HCN) content at different stages resulted in the identification of varieties IS-4776, 3247, 2944, G-10-2, 1059, 6128, L-309, 2194, T-



30, NS 256 and T-26 among single cut and piper, NKT-2 and 334 among multicut strains, low in HCN content at all the stages of plant growth. Among these, 1059, 3247 and NS 256 were found to be least responsive to changes in environment, as these had 'b' values less than one. The results are being used to breed low HCN lines of forage sorghum<sup>14</sup>.

The content of tannin, another toxic constituent in the seeds of forage sorghum, varied from 0.1 to 6.3%, while in the fodder samples it varied from 3.00 to 29.8 mg/g as catechin equivalent. It was established that varieties of forage sorghum with higher tannin content were lower in digestibility compared to varieties having low tannin content.

Diallel analysis revealed that the tannin content was governed more by non-additive genetic variance, though additive genetic variance was also present to a sizeable extent. The character was found to be highly heritable. *S. caudatum* (having high tannin content) proved to be a poor combiner, whereas *S. roxburghii* (having low tannin content) proved to be a good combiner. It was found that low tannin was dominant over high tannin content and thus breeding for low tannin appeared to be feasible, since varieties with low tannin were also identified<sup>4</sup>.

Male sterile lines good for both yield and quality have been identified and are being utilized in hybridization programme throughout the country<sup>15</sup>.

For the first time, information with regard to variability in quality parameters of all the forage germplasms has been obtained; this has opened up new avenues for better exploitation of forages for yield and quality.

In forage sorghum, six different species of *Eu-Sorghum* were selected primarily on the basis of their nuclear DNA content and morphological variation among them. Heterosis of the order of 206.42% over better check was obtained in respect of green fodder yield in cross *S. caudatum* × *S. roxburghii*. For dry fodder yield, it was maximum (228.43%) in the case of *S. durra* × *S. roxburghii*. A good combining species, namely *S. roxburghii*, having low ADF and tannin content, was identified and is being used for the improvement of quality in forage sorghum<sup>16</sup>. *S. roxburghii* and PJ7R were identified to be resistant to leaf spot diseases. PJ7R is also low in tannin and is being used in breeding forage sorghums with low tannin content. The recently evolved variety S-136 has blood of PJ7R and, therefore, has low tannin content.

Experiments comprising various crop rotations, including annual as well as perennial crops, showed that Napier × Bajra hybrid intercropped with berseem in winter and with cowpea during summer gave maximum yield (1700-2000 q/ha) per year. The other rotations of Napier × Bajra hybrid + Lucerne also gave encouraging results. Among the annual crop rotations, sweet Sudan grass-berseem yielded 1500-1700 q/ha of green fodder per year. For areas having limited water supply, the rotation bajra + cowpea-jowar + cowpea-oats was considered to be most suitable. It was further observed that by adopting all the three rotations, one can ensure regular supply of forage throughout the year<sup>17</sup>.

Forage crops, in general, are shy in seed setting. However, our work on this aspect gave good results. It has been observed that in sorghum, good seed yields could be obtained by sowing at appropriate time

(first fortnight of June) and adopting proper plant protection measures, particularly against the midge. The work done on SSG 59-3 has indicated that by planting it in the month of April, one successful cutting for fodder could be obtained; the regenerated crop gave equally good seed yield. Ratoon crop of SSG 59-3, which starts regenerating in February-March, gave much better seed yield compared to the main season crop. The seed thus obtained is healthier compared to that obtained from the main season crop. This practice is now commonly used for seed production in Sudan grass.

#### (4) Arid Crops, Grasses, Trees and Shrubs

Two promising varieties of guar developed have been released. Basic researches have revealed that in these, relatively less genetic variability existed for endosperm, gum and protein content. Increase in seed yield and size could, therefore, be the immediate aims to improve gum productivity per unit of area. Stable genotypes for yield and gum content were identified for the first time. Plant type concept in guar was also evolved<sup>18</sup>.

Rosha grass has been found to be extremely suitable for the production of aromatic oil under irrigated conditions in dry parts of Haryana. Similarly, *Isabgol* has been found to perform well in western parts of Haryana, specially under poor management conditions.

Work on forage yield and quality parameters of *Sewan* grass (*Laisurus indicus*) and *anjan* grass (*Cenchrus ciliaris*) revealed that the former was better in yield as well as quality for a

longer duration, as it retained higher protein and digestibility values (IVDMD) compared to the latter.

Work on genetic improvement in aak (*Calotropis procera*)<sup>1</sup>, thumba (*Citrullus colocynthis*) and *Kheiri* (*Prosopis cineraria*) was taken up for the first time; some useful findings were obtained. Genetic variation in *Kheiri* revealed that some selected elite lines can be as fast growing as *Acacia tortalis*.

Work on jojoba, guayule, koo-babool, saff bush and candelila wax plant has been started both at CAZRI and HAU since 1977<sup>19</sup>.

Work on rangeland management involving animal component for studies on milk and meat production, silvi-pastoral management involving fodder-cum-fuel tree species, and evaluation of grasses and legumes for their feeding value was initiated when Paroda was at CAZRI, Jodhpur<sup>20</sup>.

Two books<sup>21,22</sup> edited jointly by Paroda and S K Arora have been published.

#### Selected Publications

1. Paroda R S, Importance of synchrony of ear emergence in plant breeding programme, *Nature, Lond.*, **223**(5318) (1971) 351-52.
2. Paroda R S & Hayes J D, An investigation of genotype-environment interactions for rate of ear emergence in spring barley, *Heredity*, **26** (1971) 157-76.
3. Hayes J D & Paroda, R S, Parental generation in relation to combining ability analysis in spring barley, *Theor appl Genet*, **44** (1974) 373-77.
4. Paroda R S, Saini M L & Arora S K, Inheritance of tannin content in *Eu-Sorghum*, *Z Pfland*, **74** (1975) 251-56.
5. Paroda, R S & Rees H, Nuclear DNA variation in *Eu-Sorghums*, *Chromosoma (Berl)*, **32** (1971) 353-63.



6. Paroda R S, Chaudhary B S & Solanki, K R, Reciprocal differences in maize  $\times$  teosinte hybrids, *SABRAO Newslett*, **5** (1973) 59-61.
7. Dangi O P & Paroda R S, Studies on stability parameters in forage sorghum, *Forage Res*, **4** (1978) 7-15.
8. Paroda R S, Karwasra R R & Yadav H R, Prediction of phenotypic performance in fodder cowpeas. Proc 2nd General Congress SABRAO, New Delhi, 1973, *Indian J Genet*, **34A** (1974) 1148-56.
9. Karwasra R R, Yadav H R & Paroda R S, Prediction of phenotypic performance through genotype-environment interaction studies in sweet clover (*Melilotus paryiflora* Desf), *Euphetica*, **24** (1975) 261-67.
10. Paroda R S, Breeding of cultivated forages for improved yield and quality, Proc Symp Strategy and Experimental Approaches for Genetic Improvement of Forages for Increased Animal Productivity, *Indian J Genet*, **37** (1977) 304-10.
11. Paroda R S, Leafiness—An important criterion for improvement in yield and quality of forages, *Forage Res*, **1** (1975) 145-49.
12. Chaudhary B S, Paroda R S & Solanki K R, A new crossing technique in clusterbean (*Cyamposis tetragonoloba* (L.) Taub), *Curr Sci*, **43** (1974) 456-59.
13. Dangi O P, Paroda R S, Kishore C & Solanki K R, Crossing technique for enhanced seed setting in oats (*Avena sativa* L.), *Forage Res*, **2** (1976) 73-76.
14. Paroda R S, Breeding of cultivated forages for improved yield and quality, Proc Symp Strategy and Experimental Approaches for Genetic Improvement of Forages for Increased Animal Productivity, *Indian J Genet*, **37** (1977) 304-10.
15. Paroda R S, Diallel analysis for plant height in wheat, *Cereal Res Commun*, **2** (1974) 147-58.
16. Saini M L & Paroda R S, Combining ability analysis for forage attributes in *Eu-Sorghums*, *Indian J Genet*, **37** (1977) 463-69.
17. Paroda R S, Improvement and management of cultivated fodder for increased animal productivity, Proc International Symp on Arid Zone Research and Development, 1978, edited by H S Mann (CAZRI, Jodhpur) 1980, 311-16.
18. Rao G V S & Paroda R S, Factor analysis in clusterbean (*Cyamposis tetragonoloba* (L.) Taub.), *Theor appl Genet*, **62** (1982) 273-76.
19. Paroda R S, Plant resources of Indian arid zone for industrial uses, *Proc International Arid Lands Conference on Plant Resources*, edited by J R Goodin and D K Northington (Texas Tech Univ, Texas), 1979, 261-81.
20. Paroda, R S & Mann H S, Rangeland management for increased primary and secondary productivity in Indian arid zone, *Proc International Arid Land Conference on Plant Resources*, edited by J R Goodin and D K Northington (Texas Tech. Univ, Texas) 1979, 661-77.
21. *Guar—Its improvement and management*, edited by R S Paroda and S K Arora (Indian Society of Forage Research, Hissar), 1978, pp 169.
22. *Forage sorghum—Its improvement, management and utilization*, edited by R S Paroda and S K Arora (Indian Society of Forage Research, Hissar) 1982, pp. 213.

## N Parthasarathy

Early studies on cytology of rice emanated from Japan and India. The first significant record is the obtaining of a haploid from one of the twin seedlings isolated from GEB 24 variety at Coimbatore. This method was used by Dr Muntzing of Sweden to obtain haploids from other cereals. The importance of haploid is through their utilization to get homozygous diploids (purelines) and this will accelerate the traditional breeding methodology. This involves laborious field technology and is of rare occurrence; recent advances in pollen culture *in vitro* have simplified the technology.

Mutants varying in height, grain size and shape, chlorophyll deficiencies, leaf characters and semisteriles were obtained from  $X_2$  generation of X-irradiated seeds of GEB 24 for the first time in rice in the early thirties. A semisterile plant was identified as a translocation heterozygote and its progeny consisted of normal, semisterile and diminutive dwarfs. The occurrence of dwarfs was presumed to have arisen through mutation at the point of interchange and not through position effect. In this connection, Dr Ake Gustaffson and Ivor Gadd [*Hereditas*, 55 (1966) 310] have also confirmed that the occurrence of dwarf is not due to position effect.

Cytological analysis of two more X-irradiated derivatives has indicated that

one of them was a primary trisomic and the other a secondary trisomic. The importance of trisomics has been recognized and recent advances relate to the identification of the chromosomes of the haploid set with the corresponding 12 linkage groups of rice; this approach is a useful adjunct in genetical analysis in the mapping of genes in each of the chromosomes.

The short culm mutants were also isolated and these gave better yields in fertile soils. However, these were discarded, as artificial fertilizers were not in vogue during the thirties and the farmers wanted more straw for the draft animals. The role of semidwarfs in ushering in the green revolution in rice needs no comment.

Cytotaxonomic studies on the tribe Phalarideae, which includes six genera, have indicated that three of these, viz., *Ehrharta*, *Micraolena* and *Tetrarrhena*, should come under *Oryzae*, clarifying and lending support to the findings of early systematists. In reviews on rice breeding, it has been indicated that the introduction of gene or genes for non-sensitivity to photoperiod changes in the rice varieties will facilitate adaptability to wider ranges of latitudes as well as seasons and that these can be fitted in crop rotation to enhance the total productivity in the same area.



## Sugarcane

The finding that the indigenous sugarcane of North India, *Saccharum barberi* and *S. sinense*, have arisen through promiscuous and natural hybridization between *S. officinarum* and *S. spontaneum* in the region of Bengal, Bihar and Orissa has gained international recognition. To quote Ernst Artschwager and E W Brandes (*USDA Agricultural Handbook No. 122, 1958*) 'For many years Barber believed the slender cultivated canes of northern India to have been derived from the indigenous *S. spontaneum*. It was left for Parthasarathy, in the light of cytological and taxonomic evidence accumulated since 1920 and the concept of tropical noble cane origin since 1928, to construct an ingenious and acceptable explanation of subtropical cane origin, namely the promiscuous natural hybridization of tropical noble (*S. officinarum*) and indigenous subtropical (*S. spontaneum*) forms. In the selective processes used by the early agriculturists, the hybrids were singled out as better suited to the rigorous climatic conditions northward and became predominant there. In a later brief publication on the origin of *S. officinarum*, Parthasarathy added cytological evidence to show that *S. officinarum* may have basic complement genes in common with other close relations of the Andropogoneae widely distributed in India. This relationship is obviously quite possible and can be readily reconciled with our theory of *S. officinarum* origin'.

They believed that *S. robustum*, a wild cane variety collected from New Guinea, was the progenitor of cultivated sugarcane, *S. officinarum*, but it was suggested by Parthasarathy that it was

quite possible that these wild canes have also arisen through hybridization with *S. officinarum* and *S. spontaneum*. This suggestion has found support from Daniels *et al.* [quoted by Babu C N, *Sugarcane* (Allied Publishing Co, New Delhi) 1979, 47] who have mentioned that the description of flavanoid pattern would support the suggestion of Parthasarathy that most of the *S. robustum* forms arose through natural hybridization of *S. officinarum* and *S. spontaneum* in the region of Pacific Islands in the same way as *S. barberi* originated in India.

The five varietal groups of *S. barberi*, each with a different chromosome number, classified by Barber, created difficulties as to their origin. But such different chromosome numbers could have arisen from the natural hybrids of *S. officinarum* and *S. spontaneum* by chromosome elimination through the formation of split spindles, as has been postulated and verified through cytological analysis.

*S. officinarum*, with  $2n = 80$  chromosomes, forms a homogeneous group and the inferred basic number,  $x = 10$ , brings it into line with the simpler members of the Andropogoneae. It is generally difficult to trace the relationship between high polyploid species by cytological analysis of the hybrids. This difficulty was overcome by reducing the *Saccharum* complement by hybridization and repeated back-crossing of *S. officinarum* with comparatively lower forms like *Sclerostachya fusca* ( $2n = 30$ ). Cytological analysis of these back-cross hybrids has now indicated that the inferred basic number,  $n = 10$ , of *S. officinarum* is composed of two different chromosome complements of  $x = 5$  each and one of

these is common to both *officinarum* and *Sclerostachya*.

The heteroploid chromosome numbers of *S. robustum* indicate a probable origin similar to that of the North Indian canes. C A Barber [*Mem Dep Agric India, Bot Ser*, **7** (1915) 1-106] claimed *Katha*, the thinnest type of North Indian canes, as having originated from *S. spontaneum* on the basis of morphological characters. It is quite possible that *S. robustum* is another extreme showing more affinity to *S. officinarum*, though it is presumably of similar ancestry, with the difference, however, that conscious selection in the sub-tropics resulted in the origin of the North Indian canes, while in the equatorial region, natural selection perhaps favoured the establishment of canes like *S. robustum*.

What is required, therefore, is concentration of cytogenetical studies on these lower-chromosome forms. Investigations will have to be taken up to identify the two fundamental basic chromosome complements of  $x = 5$  each of *S. officinarum* with the basic complements of the lower-chromosome forms of *Erianthus*, *Sorghum* and other grasses.

## Selected Publications

1. Parthasarathy N, Ramiah K & Ramanujam S, A haploid plant in rice, *J Indian bot Soc*, **13** (1934) 153-64.
2. Parthasarathy N, Cytogenetical studies in Oryzeae and Phalarideae. I. Cytogenetics of X-ray derivatives in rice, *J Genet*, **37** (1938) 1-40.
3. Parthasarathy N, Cytogenetical studies in Oryzeae and Phalarideae. III. Cytological studies in Phalarideae, *Ann Bot New Ser*, **3** (1939) 43-76.
4. Parthasarathy N, X-ray mutation in rice, *Nature, Lond*, **143** (1939) 340.
5. Parthasarathy N, The probable origin of North Indian sugarcane, *J Indian bot Soc (MOP Iyengar Comm Vol)* (1946) 133-50.
6. Parthasarathy N, Origin of noble cane *Saccharum officinarum*, *Nature, Lond*, **161** (1948) 608.
7. Parthasarathy N, Chromosome elimination in *Saccharum*, *Nature, Lond*, **168** (1951) 383.
8. Parthasarathy N, Some cytogenetical aspects of the origin of sugarcane, *Indian J Genet Pl Breed*, **59** (1952) 63-66.
9. Parthasarathy N, The need for rice varieties with wide adaptability, *Int Rice Comm Newslett*, **8** (1959) 20-25.
10. Parthasarathy N, Future trends in the development of new varieties of rice, *Int Rice Comm Newslett*, **16** (1967) 32-40.
11. Parthasarathy N, International approach to problems of rice breeding, *Indian J Genet Pl Breed*, **3** (1970) 531-45.
12. Parthasarathy N, Rice breeding in tropical Asia up to 1960, in *Rice breeding International Rice Research Institute, Los Banos, Philippines* 1972.



## Prem Narain

### (1) Theoretical Statistical Genetics

The research contributions under this head pertain broadly to three areas: genetic properties of population, stochastic processes in population genetics and genetics of quantitative variability.

(i) *Genetic properties of population* : With respect to a single locus with two alleles, the coefficient of inbreeding of an individual is defined as the probability that the two genes possessed by that individual at the locus are identical by descent. This approach was generalized for an arbitrary number of linked loci and the effects of linkage on the homozygosity of a selfed population as well as of a population under mixed selfing and random mating were studied<sup>1</sup>. In the context of genetic incompatibility, heterozygote  $\times$  homozygote mating systems were generalized to situations in which several loci are segregating independently. The form of Fundamental Theorem of Natural Selection due to Fisher depends on the presence or absence of dominance. It was shown that in case of dominance due to scale effect, the two forms of the theorem would be identical.

(ii) *Stochastic processes in population genetics* : Original researches were made in the field of stochastic process as applicable to population genetics. Citations

of this work appeared in the papers of Cockerham, Kimura, Aase, Nei, Maruyama, Watterson, Karlin, Tavaré and several other workers as well as in the books "Introduction to population genetics theory" by J F Crow and M Kimura ; "Evolution and genetics of populations, Vol. 2, Theory of gene frequency" by S Wright; and "Molecular population genetics and evolution" by M Nei. In particular, genetic phenomena were modelled by diffusion and Markov chain methods. A new concept of the distribution of time taken for the fixation of a gene in finite populations was introduced<sup>2</sup>. This concept has since found useful applications in evolution and animal breeding. The problem of survival of recessive lethals in finite populations, which led to the development of a new diffusion process with killing component, was studied<sup>3</sup>. Subsequently, a new mathematical method of conditioned diffusion equations in relation to population genetic problems, which are parallel to the Fokker-Planck equations used in physics, was developed<sup>4</sup>. Based on similar considerations, conditional Markov chains were also used. The fixation probability of a gene under random fluctuations in selection intensities in a small population using Markov chain methods was determined<sup>5</sup>. Further, using diffusion approach, a theory which could give the average age of a mutant at a particular frequency conditional to its

---

Director, Indian Agricultural Statistics Research Institute, New Delhi-110012; Residence : Director's Bungalow, IASRI, Library Avenue, New Delhi-110012.

eventual loss from the finite population was developed. The statistical properties of the conditional equilibrium distribution under steady flux of mutation were studied subsequently using the diffusion approach<sup>6</sup>. Revised estimates of the average number of heterozygous nucleotide sites in mammals made using this approach were found to be lower and close to the observed values than those obtained by Kimura, in which the underlined stochastic process was not conditioned. The polymorphism and evolution of the Rh blood groups for first arrival probability and arrival time were studied using diffusion methods<sup>7</sup>. Several of the results obtained in this field are given in my Presidential Address to the Section of Statistics of the 70th Indian Science Congress held at Tirupati (A P) in January 1983 under the title "Stochastic problems and methods in population genetics"<sup>8</sup>.

(iii) *Genetics of quantitative variability*: The effect of three alleles at each locus in describing continuous variation was examined by extending the mathematical representation of laws of genetics to multiple allelic systems<sup>9</sup>. A general procedure was discussed for the problem of optimum group size in a progeny testing programme where the aim is to minimize the cost of the programme at a given rate of genetic improvement. The concepts of heritability and expected response to selection were generalized for a multi-character situation. A new corrected daughter average index for selecting dairy sires using correction for an auxiliary trait was developed. The use of auxiliary traits in combined selection for poultry improvement was examined<sup>10</sup>. Designs for partial diallel crosses for use in plant breeding were constructed<sup>11</sup>.

## (2) Applied Statistical Genetics

These contributions are grouped, according to the species of application, into four heads, viz., *Drosophila*, plants, animals and man.

(i) *Drosophila*: In the research project 'Fundamental study of quantitative inheritance', *Drosophila* was used as a tool with the objective of developing models for the inheritance of quantitative characters for ultimate use in large animal selection experiments. Results on the response to selection for fecundity in *Drosophila melanogaster* and on the application of diallel crosses in such studies were reported. Subsequently, results on the estimation of components of variation due to interaction between genotype and temperature for wing length and bristle number were reported.

(ii) *Plants*: With the help of data on  $7 \times 7$  diallel cross in wheat and  $8 \times 8$  diallel cross in *triticale*, statistical techniques for analysing complete and partial diallel crosses involving several characters were investigated and the use of discriminant function in such studies was demonstrated.

(iii) *Animals*: Under this head, the contributions are further sub-grouped into (a) cattle and buffaloes, (b) sheep, and (c) poultry.

(a) *Cattle and buffaloes*: With the help of data on Indian cattle collected from different organized farms in the country, it was found that the genetic gain in the first lactation milk yield could be increased by 3-11% if selection is based on a 'phenotypic index' in which the main trait is expressed as deviations from the expected values predicted with the help of one or more auxiliary traits<sup>12</sup>. A new sire index based



on this technique was found to be superior to others when applied to data on Sahiwal breed of Indian cattle. Statistical techniques were used for separating genetic from environmental trends using records maintained in dairy herds over several generations of selection. Some aspects of yield-survival relationship in dairy cattle were studied. The efficiency of indirect selection for life-time production was also studied. A plan for evolving a dairy breed making use of animals of different grades due to crossing of Friesian bulls with Sahiwal cows, available at Military Dairy Farms in India, was prepared. A proposal for undertaking cross-breeding among three important breeds of Indian buffaloes to combine the desirable characters of milk production and fat percentage was made and a corresponding breeding plan developed. Using the data on different grades of cross-bred animals available at Military Farms, the optimum level of exotic inheritance for stabilizing the breed was studied in relation to milk production and calving interval. Lactation performance indices in Sahiwal and Haryana cattle were constructed and studied by maximizing the variation for the index between animals relative to the within animals. A series of studies on Sahiwal cattle and Murrah buffaloes located at Chak-Ganjaria Farm at Lucknow, were undertaken. Investigations were undertaken to study the association between the immunogenetic traits, such as blood type and economic traits, such as milk yield in cattle and buffaloes, so that the performance of an animal could be predicted on the examination of its blood type and the decision for the selection of the animal could be made at any early stage.

(b) *Sheep* : With the help of data collected at the Sheep Breeding Farm

under the scheme for improvement of sheep and wool by crossing Kashmiri ewes with Rambouillet rams, an investigation on the relationship between the retention of a sheep in the flock and its wool yield in the initial clip was undertaken. With the help of the same data, the use of discriminant function and  $D^2$ -statistic in a cross-breeding programme with sheep was demonstrated. For dealing with the case of unequal variance-covariance matrices with such data, an alternative linear procedure which minimizes the probabilities of misclassification and is a minimax procedure was later used.

(c) *Poultry* : The Government of India, in collaboration with the state governments, initiated a series of Coordinated Poultry Breeding Programmes for improvement of egg production in the country. This involved evolving a strain of poultry with high level of egg production by selecting birds on the basis of Osborne's index which combines in an optimum way, the information on the individual bird with the average performance of the sire and dam families to which the bird belongs. The Regional Poultry Farm at Bhopal which initiated this breeding experiment collaborated with me for operating this programme for about seven years during 1972-1979. With the data so collected, the response to selection for rate of lay was studied. Hatch and pen effects on some performance traits in White Leghorn were investigated.

(iv) *Man* : In human nutrition, one talks of either genotypic or environmental variance ignoring the interaction between the two. However, intra-individual variation in protein or energy intake of an individual is found to vary over time and to persist even when data are averaged over

a week. A new genetic model for such studies was, therefore, developed emphasizing the importance of the covariance term in the variance of the sum of genotypic and environmental effects over time in the same individual within the framework of ontogenic growth. Using such a model, the genetic significance of intra-individual variation in energy requirement and of the auto-regulatory mechanism in nitrogen balance was studied<sup>13</sup>.

### Selected Publications

1. Narain P. Homozygosity in a selfed population with an arbitrary number of linked loci, *Genetics*, **59** (1966) 254-66.
2. Narain P, A note on the diffusion approximation for the variance of the number of generations until fixation of a neutral mutant gene, *Genet Res*, **15** (1970) 251-55.
3. Robertson A & Narain P, Survival of recessive lethals in finite populations, *Theoret Pop Biol*, **2** (1971) 24-50.
4. Narain P, The conditioned diffusion equation and its use in population genetics, *J R Stat Soc, Lond*, **B36** (1974) 258-66.
5. Narain P & Pollak E, On the fixation probability of a gene under random fluctuations in selection intensities in small populations, *Genet Res*, **29** (1977) 113-21.
6. Narain P, On the statistical properties of the conditional equilibrium distribution under steady flux of mutations, *Proc Indian natn Sci Acad*, **B45** (1979) 239-46.
7. Nei M, Li WH, Tajima F & Narain P, Polymorphism and evolution of the Rh blood groups, *Jap J hum Genet*, **26** (1981) 263-78.
8. Narain P, Stochastic problems and methods in population genetics, *Residential Address, Section of Statistics, 70th Indian Science Congress*, Pt II (1983) 1-32.
9. Narain P, The description of gene action and interaction with multiple alleles in continuous variation, *Genetics*, **52** (1965) 43-53.
10. Narain P, Malhotra P K & Wahi S D, The use of auxiliary traits in combined selection for poultry improvement, *Indian J Poultry Sci*, **18** (1983) 37-47.
11. Narain P & Arya A S, Truncated triangular association scheme and related partial diallel crosses, *Sankhya*, **B43** (1981) 93-103.
12. Narain P & Mishra A K, Efficiency of selective breeding based on a phenotypic index, *J Genet*, **62** (1975) 69-76.
13. Sukhatme P V & Narain P, The genetic significance of intra-individual variation in energy requirement, in "*W G Cochran's Impact on Statistics*", edited by PSRS Rao & J Sedransk (John Wiley & Sons, New York), 1984.
14. Narain P, Bhatia V K & Malhotra P K, *A handbook of statistical genetics* (IASRI, New Delhi) 1979, pp. 365.



## S S Prihar

Prihar has been working on efficient use of soil-water and fertilizer resources for crop production and maintenance of environmental quality. His important contributions are summarized below.

### **(1) Simultaneous Solute and Water Movement**

Prihar conducted elaborate studies on the displacement of non-interacting solutes as a function of quantities and rates of application of water, initial wetness of soil and redistribution time involving soils of different textures and layering sequences. The solution of the existing solute-transport equation for predicting solute distribution in the profile as a function of water movement was extended to include the initial salt concentration (of the same species) in the soil and that of the invading water. Salt distribution predicted by the extended analysis gave an excellent fit with the observed solute profiles.

These studies have enhanced our understanding concerning the role of distribution and discontinuities of soil pores in conducting salt and water. When infiltration plus redistribution times were matched, a given quantity of water applied at faster rates had moved the salt deeper in silt loam and sandy loam soils, while the reverse was true for loamy sand. Studies with layered system brought out that a lower layer of slightly finer pores than the

upper layer was more effective in draining the top layer than a layer which had much finer pores, e.g. a layer of loamy sand over sandy loam exhibited lower water content than that over silt loam. The results of these studies will help improve our management of mobile nutrients for crop production and avoid groundwater pollution.

### **(2) New Methods of Soil Physical Determinations**

Using the simple volume-weight relationships, Prihar developed inexpensive and rapid methods for determining water content and air porosity of soil and bulk density of soil clod. The 'soil moisture gauge' developed by him fetched him a cash prize of the Inventions Promotion Board. It has been patented. He has also developed simple techniques for measuring the force of emerging seedlings and pressures of growing roots.

### **(3) Moisture Conservation by Tillage**

Evaporation reduction by shallow tillage had been a matter of controversy for a long time. Through his elaborate experiments conducted under controlled conditions, Prihar has reconciled this controversy. He has shown that the role of tillage-induced mulch in moisture conservation is governed by the combined effects of soil-type, atmospheric

evaporativity ( $E_0$ ), time and depth of tillage and porosity of soil mulch. Tillage reduces evaporation on all soils under low evaporativity, but under high evaporativity, the benefit is restricted to finer textured soils only. He and his associates have given finite difference procedures for computing progressive evaporation losses and periodic soil moisture profiles from a knowledge of the initial moisture profile, soil water content in the shallow surface layer at two conjunctive times and  $D(\theta)$  and  $K(\theta)$  relations. They have also reported simple equations for computing vapour loss through dry soil mulch of varying thickness and porosity within a range of wind velocity. These findings would help in making proper choice of tillage practices for moisture conservation on a given soil under given  $E_0$ . Quantitative relations between depth of water table and evaporation at the surface of sandy loam soil have also been derived.

#### (4) Economy of Irrigation Water

Prihar conducted elaborate field studies on the optimization of irrigation schedules for crops and use of straw mulch for economizing irrigation water with both adequate and limited water supplies.

(a) *Irrigation scheduling* : Under adequate water supply situation, the objective is to achieve potential yield and avoid wasteful losses of water. Prihar has given an innovative and highly practical approach for irrigation scheduling. According to this, a specified amount of irrigation, IW (based on pre-determined permissible soil water depletions) is applied after the net evaporation from USDA class A pan, since previous irrigation cumulates to a certain fraction of IW. Using this approach of IW/PAN-E ratio, Prihar and his colleagues have developed irrigation

schedules for several crops, which economize water without reducing yield compared with the existing recommendations. More recently, he and his student have used simulation approach to compute water uptake by crop from different layers and to apportion it into transpiration and evaporation. Transpiration during different periods of growth is related to yield through multiplicative models. This process permits the choice of best alternatives (in terms of aliquots and timings of water application) for optimizing yields with available water supplies. He has also provided considerable experimental data on the use of limited water for optimum crop production.

The contribution of groundwater to ET by crop as affected by depth of water table has been worked out and threshold water table depths defined for wheat and maize. The threshold water depths which cause salinization of root zone under variable composition of groundwater have also been investigated.

(b) *Straw mulching* : With a view to utilizing the surplus crop residues, Prihar conducted intensive studies on the effects of straw mulch on emergence, growth and yield of both irrigated and dryland crops. It has been shown that wheat and paddy straw can be used as a mulch to increase dry matter and yield of crops or economize irrigation or both. In irrigated summer crops, mulch benefited growth by reducing soil temperature and direct evaporation from soil, while under dryland conditions it benefited the post-rainy season crops by conserving more water in the profile, particularly in the upper soil layers. For the same yield, the mulched crop required considerably less irrigation



than the unmulched crop. Water use efficiency was considerably higher in the mulched compared with unmulched crop.

### (5) Fertilizer Use in Dryland Crops

Field experiments conducted by Prihar and his associates since 1970 have shown that where crop water supplies are limited, as is the case in rainfed crops, fertilizer rates need to be rationalized in relation to available water supplies. It has been shown that fertilizer nitrogen increases yields not only by eliminating nitrogen deficiency but also by increasing water use by the crop by stimulating greater and deeper root development. More recently, he has reported a procedure to rationalize fertilizer-N rates in relation to available water supply and nitrate-N content of the root zone. His work on fertilizer use for rainfed crops was chosen by the Fertilizer Association of India for an 'excellence in research' award on this subject.

He has also reported on suitable crop sequences of dryland crops for different soil types, potentialities for harvesting rain water and recycling it for limited irrigation.

### (6) Soil Environment and Root Development

Prihar conducted elaborate studies on root anatomy and morphology, depth of rooting and root distribution as affected by deep tillage, post-planting shallow tillage, mulching, time of wetting with limited water, pre-sowing moisture profiles and post-sowing irrigation schedules and dense traffic pans in the root zone. Through these studies, he has shown that root systems of crops can be trained by soil manipulations like mulching, tillage and water management. He has also established a root museum where

mounted specimens of root systems of several crops collected by block washing using pin-board technique are placed.

Prihar contributed invitational papers to international conferences on Role of soil physical conditions and crop production at IITA, Ibadan; Physical aspects of soil management in rice-based cropping systems at IRRI, Los Banos; to 12th International Congress of Soil Science, to Golden Jubilee Bulletin of the Indian Society of Soil Science; to Indo-US workshop at CAZRI, Jodhpur and many national meetings and workshops.

Prihar has won many national awards, including the Rafi Ahmed Kidwai Memorial Award.

### Selected Publications

1. Prihar S S, & Sandhu B S, A rapid method of soil moisture determination, *Soil Sci*, **105** (1968) 142-46.
2. Prihar S S, Singh Bhajan & Sandhu B S, Effect of soil and climatic environments on evaporation losses from mulched and unmulched pots, *J Res*, **V** (1968) 320-28.
3. Bansal S P, Gajri P R & Prihar S S, Effect of mulches on hydrothermal regime of soil and growth of maize and bajra, *Indian J agric Sci*, **41** (1971) 467-73.
4. Chaudhary T N, Bhatnagar V K & Prihar S S, Growth response of crops to depth and salinity of ground-water and soil submergence. I. Wheat, *Agron J*, **66** (1974) 350-55.
5. Prihar S S, Gajri P R & Narang R S, Scheduling irrigation to wheat using pan evaporation, *Indian J agric Sci*, **44** (1974) 567-71.
6. Ghuman B S, Verma S M & Prihar S S, Effect of application rate, initial soil wetness and redistribution time on salt displacement by water, *Soil Sci Soc Am Proc*, **39** (1975) 7-10.
7. Prihar S S, Khera K L, Sandhu K S & Sandhu B S, Comparison of irrigation schedules based on pan-evaporation and growth stages in winter wheat, *Agron J*, **68** (1976) 650-53.
8. Khera K L, Khera R, Prihar S S, Sandhu B S & Sandhu K S, Mulch, nitrogen and irrigation

- effects on growth, yield and nutrient uptake of forage corn, *Agron J*, **68** (1976) 937-41.
9. Gill K S, Jalota S K, Prihar S S & Chaudhary T N, Water conservation by soil mulch in relation to soil type, time of tillage, tilth and evaporativity, *J Indian Soc Soil Sci*, **25** (1977) 360-66.
10. Verma H N, Prihar S S, Singh Ranjodh & Singh Nathu, Yield of sub-humid rainfed crops in relation to soil water retention and cropping sequence, *Exptl Agric*, **14** (1978) 253-60.
11. Prihar S S, Singh Ranjodh, Singh Nathu & Sandhu K S, Effect of mulching previous crop or fallow on dryland maize and wheat, *Exptl Agric*, **15** (1979) 129-34.
12. Ghuman B S & Prihar, S S, Chloride displacement by water in homogeneous columns of three soils, *Soil Sci Soc Am J*, **44** (1980).
13. Jalota S K, Prihar S S, Sandhu B S & Khera K L, Root distribution, water use and yield of wheat as affected by pre-sowing and post-sowing irrigation, *Agric Water Mgmt*, **2** (1980) 289-97.
14. Sandhu B S, Prihar S S & Khera K L, Sugarcane response to irrigation and straw mulch in sub-tropical region, *Agric Water Mgmt*, **3** (1980) 35-44.
15. Ghuman B S & Prihar S S, Chloride displacement by water in layered soil columns, *Aust J Soil Res*, **18** (1980) 207-11.
16. Prihar S S, Sandhu K S, Singh Y & Singh R, Effect of N rates on dryland wheat in relation to mulching previous crop or fallow, *Fertil Res*, **2** (1981) 211-19.
17. Gill K S & Prihar S S, Effect of cultivation on evaporation, *Soil Sci*, **135** (1983) 367-76.
18. Gajri P R & Prihar S S, Effect of small irrigation amounts on the yield of wheat, *Agric Water Mgmt*, **6** (1983) 31-41.
19. Gajri P R & Prihar S S, Rooting, water use and yield relations in wheat on loamy sand and sandy loam soils, *Field Crops Res*, **12** (1985) 115-32.
20. Prihar S S, Gajri P R & Arora V K, Nitrogen fertilization of wheat under limited water supplies, *Fertil Res*, (1985) 811-18.
21. Chaudhary M R, Gajri P R, Prihar S S & Khera R, Effect of deep tillage on soil physical properties and maize yields on a coarse textured soil, *Soil & Tillage Res*, **6** (1985) 32-44.
22. Jalota S K & Prihar S S, Effects of atmospheric evaporativity soil type and redistribution time on evaporation from bare soil, *Aust J Soil Res*, (1986), accepted.



## K Ramiah

After getting his diploma in agriculture from the Government Agriculture College, Coimbatore, Ramiah joined the same college as a Research Assistant in October 1914. He concentrated his attention on rice breeding and genetics. One of his early attempts was yield improvement in the early group of rice varieties. In this approach, he made selections in the early summer rice (*Kuruvai*) in Tanjore district of Madras Province. One of his selections (later released as Adt 3), because of its comparative earliness and higher yield than the bulk from which it was isolated, quickly spread and held the field in the whole of Cauvery delta covering about 2,00,000 hectares from 1920 to 1960, a period of 40 years.

In 1926, Ramiah was deputed for higher training in plant breeding and genetics at the Cambridge University. During his stay of about 2 years, he not only obtained the diploma specializing in genetics but also the MSc Degree for a piece of research in wheat. During this period, he utilized the vacation by spending three weeks at the world famous Crop Breeding Station, Swalof in Sweden and a week in Copenhagen to study the plant breeding and seed production programmes of commercial firms in Denmark. He attended the Fifth International Genetic Congress in 1927 held in Berlin.

On his return to India, Ramiah continued to work at the Government Agriculture College, Coimbatore, till his appointment as Paddy Specialist to Madras Government in 1930. He held this position for 7 years. He was instrumental in and responsible for organizing and putting rice research in Madras Presidency on a firm footing and in a most comprehensive manner with a centre for fundamental research at Coimbatore and regional research stations in important tracts with different rice growing conditions, viz., Aduthurai in Tamil Nadu, Pattambi in Kerala, Maruteru in Andhra Pradesh and Berhampur in Orissa.

In his attempts to increase rice yields through varietal improvement in association with his colleagues, of whom there are many, he was able to identify over 30 improved varieties of rice, developed through hybridization or selection process. In his endeavour, his personal contribution has been around a dozen cultivars; the rest were due to collaborative efforts. A notable example is the variety "GEB 24", the cultivation of which spread to various parts of India and even to countries outside India (sometimes with a different name). This variety has been effectively used as a parent in hybridization work at several centres. Even today, it commands a premium price in the market. The highly useful blast

---

Formerly, Director, Central Rice Research Institute, Cuttack; Residence : Swathi, 108, 8th Main Road, 19th Cross, Malleswaram, Bangalore-560055.

resistant variety, Co 25, was the outcome of interdisciplinary approach. Co 25 is still being grown in parts of Tamil Nadu.

Ramiah was the first in India to initiate work on interspecific hybridization and induction of mutation in rice through X-ray irradiation. Some of these early mutants were like the modern short-statured varieties, but not in production. He maintained a large collection of varieties, valuable genetic material, including wild rices and other species of *Oryzae*. He realized the importance of germplasm collection, assessment and maintenance even at that stage.

Ramiah initiated investigation on water requirements of paddy in cooperation with the Government Irrigation Department and started experiments on direct sowing of rice instead of transplanting after puddling.

Ramiah's early investigations on the genetics of rice are also of considerable importance and significance. Along with Dr Kadam of Maharashtra state, he initiated work on gene symbolization in rice. He standardized description of plant parts. These are being adopted today by rice scientists.

His services were lent to the Indian Central Cotton Committee in 1937 and he was appointed Geneticist and Botanist incharge of cotton research at the Institute of Plant Industry, Indore. He made substantial contribution to cotton genetics.

It was at Ramiah's initiative that the Government of India decided to set up the Central Rice Research Institute, Cuttack. He was the founder Director of this institute for five years (1946-51). The following are among the programmes initiated by him :

(i) The practicability of growing a short duration second crop of rice in the canal irrigated area (which practice did not exist before) was demonstrated. This later became a regular practice and is adopted in thousands of hectares.

(ii) The effectiveness of chemical fertilizers was demonstrated in farmers' fields. This was yet another major step for increasing rice production in Orissa.

(iii) His idea of making a survey of the Jeypore tract of Orissa which had a wide diversity of rice varieties was considered by the ICAR and the latter sanctioned a scheme for the collection of the material and its assessment. During the survey, *Oryza perennis*, the immediate progenitor of cultivated rice, was collected along with several intermediate types arising out of natural crossing between *O. perennis* and cultivated rice. This survey proved that this area is a secondary centre for the evolution of cultivated rice varieties.

To Ramiah again goes the credit of suggesting a coordinated programme of rice breeding among the Southeast Asian countries. This involved the production of new varieties by crossing important local *indica* varieties from different countries with selected varieties from Japan. When this scheme was in progress with financial support from FAO, a similar scheme, financed by ICAR, was drawn up for the benefit of different states in India. The crossing was done at the Central Rice Research Institute (CRRI), Cuttack, and seeds from  $F_1$  plants were sent to the participating countries and to different states in the country. A major outcome of these programmes was the development of *Malinja* and *Mahsuri* in Malaysia and Adt 27 in Madras state.



Ramiah was appointed a Member of the Standing Advisory Committee (in Agriculture) of FAO in which capacity he served for two years. As Director of CRRI, his services were lent to FAO to enable him to join as a Member of a Commission set up to review the agricultural situation in Thailand and to recommend measures to increase agricultural production in that country. He was invited by FAO in 1947 to join as its Rice Expert. However, he could not accept the offer as he had already agreed to serve as the Director of the Central Rice Research Institute for a 5-year period. Later, in 1951, when the offer was renewed by FAO, he accepted and was appointed as an Expert on Rice Production and Breeding in Southeast Asia with headquarters at Bangkok. In this capacity, he visited rice growing countries and brought about an effective coordination in a programme for increasing rice production to meet the world shortage of this commodity. He helped FAO in the preparation of a "World Catalogue of Genetic Stocks".

FAO coopted him as a member in its missions to South Korea (1952) and South Vietnam (1953). He also joined a team of experts set up by the World Bank to visit Japan and to examine the economic feasibility of growing rice or other crops in lands reclaimed from the sea and from peat deposits. Ramiah, while he was with FAO, initiated international coordinated variety trials in S.E. Asian countries.

On his return to India after the FAO assignments, his services were utilized by the ICAR and other organizations. Ramiah was a member of the Technical Committee of the ICAR ever since it came into existence and served as a Member in its Board. During this period, ICAR

undertook periodical reviews of research of the institutions under its control. Ramiah served as the chairman of these reviewing committees.

Ramiah, in the capacity of Special Officer of ICAR, made a survey on rice research in all rice growing states in India and submitted a report indicating in detail as to how research could be coordinated and strengthened. He also acted as Chairman of an Expert Committee to investigate the causes for the low rice yields in Northeast India (Eastern UP, Bihar and Orissa) and how the position could be improved.

In 1960, Ramiah was appointed a Member of the Indo-American Team of the Ford Foundation to review the position of food production in India. He was also a member of the international team that reviewed the work of ICAR and put up proposals for its reorganization. He was later appointed Chairman, Advisory Committee on Rice Research. He acted as chairman of a committee set up to prepare a scheme for market classification of rice.

Outside of ICAR, Ramiah was entrusted with such review work in coffee research and extension in Karnataka and Andhra Pradesh (twice in 1961 and in 1972) and sericulture work in Karnataka, West Bengal and Bihar. He served as a Member of Coffee Research Committee. He also served as a Member of the Silk Board (two terms) and was the Chairman of its Research Committee. He has also been the chairman of a panel of agricultural scientists set up by the Government of India.

Ramiah was Vice-Chancellor of Orissa University of Agriculture and Technology, Bhubaneswar, Orissa during 1965-1968.

For the next six years (1968-74), he was a nominated member of the Rajya Sabha.

In recognition of his outstanding contributions, Ramiah was awarded the MBE in 1938, Padma Shri in 1955 and Padma Bhushan in 1970. He won the International Rice Year Medal in 1961, the Plaque of the Indian Society of Genetics and Plant Breeding in 1983, Sunderlal Hora Medal of INSA (1969). He is a founder fellow, Indian Academy of Sciences, Bangalore, National Academy of Sciences, Allahabad, and elected fellow (1942) Indian National Science Academy (formerly, National Institute of Sciences of India); and honorary member, Japanese Breeding Society. He was President, Agriculture Section, Indian Science Congress in 1941.

### Selected Publications

1. Ramiah K, *Rice in Madras—Popular handbook* (Madras Government) 1938.
2. Ramiah K, *Rice breeding and genetics—Scientific monograph* (Indian Council of Agricultural Research, New Delhi) 1953.
3. Ramiah K, Factors affecting rice production, *FAO Agriculture Development Paper* No. 45, 1954.
4. Ramiah K, Rice research in India, *Brief, FAO Rice Working Party Meeting*, Trivandrum, 1947.
5. Sethi R L, Ramiah K & Abraham T P, Manuring of rice, *ICAR Bull* No. 38, 1952.
6. Ramiah K, *Oryza in Wealth of India*, Vol 7 (Council of Scientific Research, New Delhi) 1961.
7. Parnell F R, Rangaswamy Ayangar G N & Ramiah K, Inheritance of characters in rice. I. *Memo Dep Agric Bot Ser*, Imperial Agricultural Research Institute, Pusa, 1917.
8. Parnell F R, Rangaswamy Ayangar G N, Ramiah K & Srinivasa Ayyangar C R, Inheritance of characters in rice. II. *Memo Dep Agric Bot Ser*, Imperial Agricultural Research Institute, Pusa, 1921.
9. Ramiah K, Inheritance of characters in rice. III. *Memo Dep Agric Bot Ser*, Imperial Agricultural Research Institute, Pusa, 1930.
10. Ramiah K, Jobitharaj S & Dharamalingam S, Inheritance characters in rice. IV. *Memo Dep Agric Bot Ser*, Imperial Agricultural Research Institute, Pusa, 1931.
11. Ramiah K, Inheritance of flowering duration, plant height and their genetic relationships, *Indian J agric Sci* (1933).
12. Ramiah K & Parthasarathy N, Inheritance of grain length in rice, *Indian J agric Sci, Camb*, (1933).
13. Ramiah K, Parthasarathy N & Ramanujam S, A haploid palnt in rice, *J Indian bot Soc*, **13** (1934).
14. Ramiah K, Parthasarathy N & Ramanujam S, Polyembryony in rice, *Indian J agri Sci* (1934).
15. Ramiah K & Ramasamy K, Breeding for *Piricclaria* resistance in rice, *Proc Indian Acad Sci* (1936).
16. Ramiah K, Rice breeding and genetical work in India, *Presidential Address, Indian Science Congress Association, Agric Sect*, 1941.
17. Ramiah K, Ghose R L M & Vachhani M V V, Improvement of rice in India, *Empire J exp Agric* (1952).
18. Ramiah K, Rice breeding in S Asia, *Indian J Genet Pl Breed* (1957).
19. Ramiah K, Present position of rice research in India, *Sunder Lal Hora Lecture*, 1969.
20. Engledow F L & Ramiah K, Investigations on yield in cereals. VII. A study of development and yield of wheat based on varietal comparison, *J agric Sci, Camb* (1930).



## M S Randhawa\*

In spite of his pre-occupation with heavy developmental and administrative tasks, Randhawa maintained a sustained and absorbing interest in scientific research and contributed a great deal to the understanding of the terrestrial and fresh-water algae of India. Even as a member of the former Indian Civil Service, he built up modest facilities with his own resources and carried on research on algae enthusiastically. His discoveries and contributions to the knowledge of algae of the Himalayas and Indo-Gangetic plains have had a tremendous impact on the development of Indian phycology. Over the last five decades, unlike professional scientists with well equipped and established laboratory facilities and a battery of students, he used to carry a microscope and box full of tubes and empty ink bottles when out on tour in connection with his official work. In the evenings, he used to examine the collected specimens and preserve such of those which were of interest. He spent almost all his holidays in making sketches and writing descriptions of the materials which were found to be of unusual interest. Thus, a hobby which was adopted as a pure pleasure in out-of-the-way places in UP like Almora, Fyzabad and Rae Bareilly ultimately proved to be very productive and of great scientific interest. He published 42 original papers on algae in leading scientific journals.

His particular interest was in the members of the family Zygnemaceae. His

first paper was on a new genus, *Ghosella*, published in 1934. A few months earlier in the same year it was described as *Zygnemopsis* by Transeau. The discovery of *Zygnema terrestris*, a terrestrial species of *Zygnema* by him in 1938 from Fyzabad district of UP was an important one, as it provided a connecting link between the genera *Zygnema* and *Zygogonium*. During 1937-38, he described nine new species of *Zygnemopsis* from UP and Punjab. Of these, the discovery of *Zygnemopsis minuta* is important in that it provides a link between saccoderm desmids and the Zygnemaceae.

In 1941, he described *Sirocladium* as a new genus, on the basis of a specimen collected from the Kumaon Himalayas and named it *S. kumaoense*. Two more new species of this interesting genus were discovered by him in 1956, one from Western Ghats in Maharashtra and the other described by Prof. Iyengar from Vandalur in Madras State. The two flat straight parallel plate-like chloroplasts in each cell distinguish this genus from *Sirogonium*. In all, he recorded 70 species of the genera of Zygnemaceae, including one of *Debarya* which is new, 9 of *Mougeotia*, including 1 new species, 11 of *Zygnema*, including 6 new species, 2 of *Zygogonium*, both new, 30 of *Spirogyra*, including 11 new species and 3 of *Sirogonium*, including 2 new species.

In 1930, he collected an important form of a terrestrial alga which was

---

Formerly, Vice-Chancellor, Punjab Agricultural University, Ludhiana.

\*Since deceased.

subsequently named as *Frittschiella* by Prof. Iyengar. This highly organized alga has phylogenetic significance and forms a connecting link between green algae and liverworts.

He followed the rule of collecting everything which he came across and never took anything for granted. A casual collector tends to dismiss any felt-like growth as a species of *Vaucheria*. In the case of microscopic plants like algae, one never knows what one is collecting till it is examined under the microscope. His discovery of *Zygnema terrestris* is an example of this rule.

One of his major contributions as the Vice-President of the Indian Council of Agricultural Research was the direction which he gave to the compilation of all available information on different groups of algae in the form of scientific monographs. He himself accepted the responsibility of writing a monograph on *Zygnemaceae* which was published in 1959. This monograph served as a model for others and set a good standard for the whole series. Subsequently, a series of monographs were brought out under his expert guidance on various groups of algae by Indian phycologists. This has put India on the phycological map of the world. In his monograph on *Zygnemaceae*, he described 580 species, including 8 of *Debarya*, 108 of *Mougeotia*, 8 of *Temnogametum*, 31 of *Zygnemopsis*, 100 of *Zygnema*, 289 of *Spirogyra*, 15 of *Sirogonium* and 3 of *Sirocladium*. This monograph provides an authentic source for research workers and students not only for acquainting themselves with the species and genera recorded and described in India, but also provides them with an incentive for exploring the states hitherto untouched and thus extending the frontiers of knowledge in respect of algae.

The unique combination of extensive scientific, literary and administrative experience with an unquenching dynamism, deep knowledge of human problems and a rare sense of humour made Randhawa's personality a versatile one. His contributions to agricultural development, art and urban landscape and environmental planning have made him a legendary figure.

### Selected Publications

1. Randhawa M S, *Ghosella indica* gen et sp nov, a new member of Conjugatae, *J Indian bot Soc*, **13** (1934) 11-16.
2. Randhawa M S, Genus *Anabaenothrix* and parallelism in evolution in freshwater algae, *Proc Indian Acad Sci*, **3B** (1936) 407-10.
3. Randhawa M S, Three new species of *Zygnema* from Northern India, *Proc Indian Acad Sci*, **4B** (1936) 239-45.
4. Randhawa M S, Genus *Zygnemopsis* in Northern India, *Proc Indian Acad Sci*, **5B** (1937) 297-314.
5. Randhawa M S, Observations on some new and interesting algae from Northern India, *Hedwigia*, **78** (1939) 273-83.
6. Randhawa M S, *Zygnema terrestris* Randh, from the Kumaon Himalayas, *Curr Sci*, **9** (1940) 373.
7. Randhawa M S, *Iwanoffia terrestris* (Iwan) Pascher from Fyzabad, *Curr Sci*, **9** (1940) 459-60.
8. Randhawa M S, *Zygogonium kumaoensis*, a new species of *Zygogonium* from Kumaon, *J Indian bot Soc*, **19** (1940) 247-49.
9. Randhawa M S, *Sirocladium*, a new terrestrial member of the Zygnemales, *Bot Gaz*, **103** (1941) 192-97.
10. Randhawa M S, Genus *Cyclindrocapsa* in India, *Curr Sci*, **10** (1941) 292-94.
11. Randhawa M S, A critical review of some recently created new species of Indian Zygnemales, *Proc Indian Acad Sci*, **18B** (1943) 73-81.
12. Randhawa M S, A note on two new species of *Spirogyra* and *Sirogonium*, *J Indian bot Soc*, **37** (1958) 380-81.
13. Randhawa M S, Notes on some new algae from India, *Bot Gaz*, **120** (1958) 25-31.
14. Randhawa M S, *Zygnemaceae: A monograph* (Indian Council of Agricultural Research, New Delhi) 1959.



## N S Randhawa

Randhawa is one of the outstanding soil chemists of the country and has made significant contributions in the field of micronutrient research as related to plant, animal and human nutrition. His work has resulted in the identification of widespread zinc deficiency in Indian soils. He established that continuous depletion of micronutrient in the intensive and exploitative farming systems adopted in Punjab has resulted in widespread deficiency of micronutrients, particularly zinc. His researches aimed at delineation of areas susceptible to nutrient stresses in animals and human beings are of considerable applied significance.

Randhawa has also contributed to the solution of fertility problems and nutritional disorders of fruit trees and field crops.

His researches have not only contributed to our knowledge of the chemistry of zinc and other minor elements in Indian agriculture, but have also resulted in large scale use of micronutrients, particularly zinc sulphate, for increasing production.

Randhawa and his coworkers have contributed more than 270 papers, of which 170 relate to micronutrients. His major contributions are given below in brief.

### **Delineation of Responsive Areas**

A systematic effort at delineation of micronutrient responsive areas was first initiated by Randhawa during 1953-55. Results accruing from these studies have been helpful in mapping areas of micronutrient deficiencies and associating the total and available micronutrients with different soil characteristics.

### **Response of Crops to Micronutrients**

For the first time, the response to the application of zinc was recorded in Punjab by Randhawa during 1953-54. Systematic research work carried out by him and his associates in this direction provided conclusive evidence that intensive agriculture led to micronutrient deficiencies. Spectacular responses have been achieved through the application of zinc to crops like maize, wheat, paddy and other foodgrains.

Zinc sulphate is the most efficient and the cheapest source for correction of zinc deficiencies in comparison to other zinc carriers. Soil application through broadcasting or placement below the seed was found to be the most effective approach in regions of acute zinc deficiency. In areas where this deficiency is marginal or the fixation capacity of the soil is very high, its foliar application may be

profitable. The optimum time for zinc application is prior to sowing of the crop. Mild zinc deficiency can, however, be corrected by applying zinc sulphate at the tillering stage. Leguminous crops in sequences are also conducive to the maintenance of favourable nutrient balance in the soil. Improvement of soil physical condition affected the micronutrient uptake favourably.

Work of Randhawa indicated that the residual availability of zinc applied @ 5 ppm lasts for only two crops in rice-wheat rotation, whereas in wheat-maize and wheat-groundnut rotations it continues for a period of 2-3 years.

### Critical Limits of Micronutrients

Critical limits of available sulphur, zinc and copper in soils and plants were determined. The pertinent findings are as follows :

(i) A critical limit of 10 ppm heat soluble sulphur and mono-calcium phosphate extractable sulphur can be adopted for predicting responses to sulphur application.

(ii) Dithizone and DTPA extractants are most dependable for predicting responses to zinc application.

(iii) Neutral normal ammonium acetate and 0.02 M EDTA gave the highest correlation values for responses to Cu application to wheat and maize.

(iv) Critical limits for predicting responses to Mn, Mo and B have been established.

### Latent Zinc Deficiency

Elucidation of the role of zinc in intermediary metabolism and activity of enzymes has amplified the possibility of

predicting the response of crops to zinc application in the early stage of growth. Randhawa's work has also indicated that determination of active zinc in plant tissues, ribonuclease activity and carbonic anhydrase activity determination provided dependable information.

### Nutrient Interactions

Interactions among nutrients in soils can be either synergistic or antagonistic. These interactions play a significant role in sustaining normal crop yields and maintaining the health of cattle and human-beings. Interactions of zinc-phosphorus, K with secondary and micronutrients, sulphur-molybdenum, phosphorus-molybdenum, copper-molybdenum and sulphur-selenium were studied. Heavy doses of fertilizer P decreased the availability of Zn in soil. The major sites of this interaction are in the roots and nodes of the plants.

Differentiation between inherent zinc deficiency and P induced zinc deficiency in the case of maize-wheat has become possible. Application of sulphur as gypsum in saline sodic and calcareous soils depresses the level of plant Mo by 50%. Application of copper depresses Mo intake slightly, but enhances the content of copper in plants significantly. This finding is very helpful in checking molybdenosis in cattle, because higher levels of Cu in the forage crops improve the tolerance of Mo toxicity. Sulphur application is also found to depress the selenium levels in plants.

Marked differences in responses to nitrogen, phosphorus, sulphur, molybdenum and zinc have been recorded among crop varieties. These have far-reaching implications from crop productivity and animal health points of view.



The differences among crop varieties to zinc stress can be attributed to the differences in (i) mining capacity of native zinc, (ii) absorption mechanism, (iii) nutrioperiodism, (iv) translocation of nutrients to stem and leaves, (v) maintenance of suitable elemental ratios in the plant tissues, and (vi) capacity to maintain zinc in an active state.

### **Toxicity**

Several trace elements (Mo, Se and F) are essential for human-beings and animals when present in small quantities, but prove toxic when they are present beyond certain critical limits.

A sizeable percentage of the well waters from Tosham block in Hissar district and Malerkotla block of Sangrur district contain toxic levels of fluorine in the saturation extract of soils and the sub-soil waters. The feeding of grasses and fodders raised on such soils, coupled with the use of such well waters for drinking purposes, causes fluorosis among the human and cattle populations of these areas. High Mo level in forage is known to cause severe dietary disorders and loss of general health of animals. The disease, designated as molybdenosis, is a Mo induced Cu deficiency. Fertilization of forage crops with S and Cu not only depresses the Mo concentration in such crops, but also enhances the concentration of S and Cu and thus increases the Mo tolerance by cattle.

A large number of corriedale sheep imported from Australia and located at the Animal Husbandry Department, Mattewara Farm (Ludhiana) died due to enzootic calcinosis. A critical study of this problem showed that it is attributable to potassium-induced magnesium deficiency.

### **Chemistry of Zinc in Soils**

The availability of zinc in soils is governed by the simultaneous chemical equilibria of several competing processes, such as adsorption, desorption, precipitation and chelation reactions. A unifying principle based on surface chemical reactions, precipitation, chelation and kinetic parameters, such as diffusive and convective movement of zinc from the soil to plant roots, has been developed from thermodynamic and kinetic measurements in soils. A number of parameters, such as quantity, intensity and buffering capacity of zinc in soils, have been defined and correlations derived to evaluate the nature and extent of reaction of zinc with soils.

### **Reaction Products of Zn in Soils**

The reaction products of zinc in acidic, alkaline, sodic and calcareous soils have been identified on the basis of thermodynamic calculations. A clear picture of the differential reaction, efficiency and effect of zinc application on the uptake of zinc and crop yields has emerged from an understanding of the kinetic factors which determine the movement of zinc to plant roots.

### **Movement of Zn in Soils**

The relative stability of Zn chelates in soil solutions of alkaline and calcareous soils and their efficiency as sources of soluble zinc for plants has been evaluated. Tracer studies with  $^{65}\text{Zn}$ -tagged chelates have shown that in alkaline and calcareous soils, these are more effective than zinc sulphate. The application of soluble zinc chelates greatly augments the diffusive and convective movements of zinc to plants resulting in enhanced uptake and growth of zinc sensitive crops.

The timely detection of zinc deficiency, fixation of critical limits in soils and plants, development of technology in respect of time, method and source of zinc application have resulted in additional crop productivity worth several hundred crores of rupees when based on the total area under these crops.

### Selected Publications

1. Randhawa N S, Kanwar J S & Nijhawan S D, Distribution of different forms of manganese in the Punjab soils, *Soil Sci*, **92**(2) (1961) 106-12.
2. Randhawa N S & Kanwar J S, Zinc, copper and cobalt status of Punjab soils, *Soil Sci*, **98** (1964) 403-7.
3. Randhawa N S & Broadbent FE, Soil organic matter-metal complexes: 5. Reactions of zinc with model compounds and humic acid, *Soil Sci*, **99** (1965) 295-300.
4. Randhawa N S & Broadbent FE, Soil organic matter-metal complexes: 6. Stability status of zinc-humic acid complexes at different pH values, *Soil Sci*, **99** (1965) 362-66.
5. Randhawa N S, Bhumbla D R & Dhingra D R, Role of soil and plant composition in diagnosis of citrus decline in Punjab, *J Res PAU*, **4**(1) (1967) 16-24.
6. Grewal J S, Bhumbla D R & Randhawa N S, Available micronutrient status of Punjab, Haryana and Himachal soils, *J Indian Soc Soil Sci*, **17** (1969) 27-31.
7. Pasricha N S & Randhawa N S, Available molybdenum status of some recently reclaimed saline-sodic soils and its effect on concentration of molybdenum, copper, sulphur and nitrogen in berseem (*Trifolium alexandrinum*) grown on these soils, *Proc Int Symp Soil Fert Evaln*, Vol 1, New Delhi (1971) 1017-25.
8. Pasricha N S & Randhawa N S, Interaction effect of sulphur and molybdenum on the uptake and utilization of these elements by raya (*Brassica juncea* L.), *Plant & Soil*, **37**(1) (1972) 215-20.
9. Brar S P S, Randhawa N S & Dwivedi R S, Studies on differences in maize varieties for susceptibility to zinc deficiency-chemical and biochemical indices, *Proc 7th Int Colloquium of Plant Analysis and Fertilizer Problems*, Vol 1, Hanover, FRG, 1974, 55-69.
10. Dhillon K S, Sinha M K & Randhawa N S, Organo-metallic phosphates. V. Complexation of zinc and phosphorus by humic compounds, *Plant & Soil*, **43**(2) (1975) 317-26.
11. Takkar P N, Mann M S & Randhawa N S, Effect of direct and residual available zinc on yield, zinc concentration and its uptake by wheat and groundnut crops, *J Indian Soc Soil Sci*, **23**(1) (1975) 91-95.
12. Takkar P N, Mann M S, Bansal R L, Randhawa N S & Harved Singh, Yield and uptake response of corn to zinc as influenced by phosphorus fertilization, *Agron J*, **68** (1976) 942-46.
13. Singh S P, Sinha M K & Randhawa N S, Diffusion on Zn-65 as influenced by rates of applied zinc in soils of divergent texture, *J Indian Soc Soil Sci*, **28**(3) (1980) 290-94.
14. Chand M, Bhumbla D R, Randhawa N S & Sinha M K, The effect of gypsum and organic amendments on the availability of zinc to rice and its uptake by this crop grown in a saline-sodic soil, *Proc Int Symposium on Salt-affected Soils*, Karnal, 1980.



## N G P Rao

Viewed against the background of the agricultural history of India, changes that influenced Indian agriculture during the 1960s and 1970s are perhaps the most significant ones. The rapid impact of these changes and the potential created do give us confidence that India could successfully meet its current and future needs of the most needed agricultural commodities. We have certainly been fortunate to have been participants in and witnesses to this great transformation. The transformation that began with irrigated wheat and rice soon pervaded dryland food crops like sorghum and pearl millet, commercial crops, fruits and vegetables. Changes leading towards the transformation of sorghum, the most important food crop of drylands, and sorghum-based cropping systems in India have been the major objectives of my endeavour.

### **Evolution, Performance, Adaptation, and Stability of New Hybrids and Varieties**

The attributes of tropical cultivars have been tallness, lateness, photosensitivity, low harvest indices, poor community performance, etc. In traditional agriculture, the timing of the main stages in the reproductive cycles of plants is optimized in relation to seasonal conditions through control mechanisms that are extremely sensitive to daylength and temperature. If this is so in traditional agriculture, the

design and development of productive and stable agriculture requires re-optimization of the cycles of growth and reproduction in such a way that the emphasis is on the economic product rather than on total dry matter and that the more critical phases of growth coincide with favourable periods of climate.

Most traditional *kharif* sorghums of the Deccan and the central Indian plateaus require 140 days or more to mature, while the duration of a normal rainy season is from the beginning of July to mid or late September. August usually represents the peak rainfall month. Such sorghums usually remain in the vegetative stage till the second week of October, and if rains cease earlier, yield losses are heavy. The total dry matter produced in normal circumstances may be as high as 450 g per plant, and nearly 70% of this is accumulated in the stalk. They are characterized by a single peak for the rates of growth coinciding with flowering. The behaviour of several African sorghums is similar. Some temperate sorghums, on the other hand, produce less dry matter per plant, exhibit two peaks for growth rates, coinciding with preflowering and grain-filling stages, resulting in 50:50 dry matter distribution.

Superior sorghum hybrids and varieties developed, viz., CSH-1, CSH-5, CSH-6 and CSH-9, have reduced duration (100-

110) days) and consistently yield well. The average yields range from 2 to 4 tonnes per hectare under rainfed conditions and are up to 6 tonnes under favourable conditions. The critical stages of growth—seedling, flower primordia and grain filling—coincide with periods of assured rainfall or satisfactory profile moisture status. Thus, in breeding for efficient water use for grain production, such corrections for duration, dry matter production and differentiation at optimal times of the season are essential and should constitute the first steps in modifying the traditional tropical sorghums<sup>1-6</sup>.

Our studies have also established that compared with improved varieties, hybrids do exhibit homeostatic advantages, particularly under moisture stress. The hybrids CSH-9, CSH-6, CSH-5 and CSH-1 were the highest yielders in the *kharif* tracts of the entire country and were the most widely adapted ones. The improved varieties were no doubt superior to locals in yield and adaptability, but were not comparable with hybrids. The locals were characterized by low means and high coefficients of variability. Further efforts to use risk aversion in plant breeding have indicated that yield and risk preference based rankings are closely related. Also correlated are adaptability and stability, lending support to our past breeding efforts towards genotype alteration and multilocational testing in the pursuit of low risk and high yields<sup>7</sup>.

We are now concerned with the task of incorporating greater levels of resistance to insect pests and diseases against the altered genetic backgrounds so as to confer greater levels of stability in performance besides reducing or even eliminating the use of pesticides. Our

emphasis now is on understanding and incorporating resistance to several of the insect pests and diseases together in altered genotypes<sup>8,9</sup>.

### **Genotype-Input Management Relations**

While there have been attempts to orient breeding towards changes in agronomy, such as mechanized agriculture, it is now the genotype change that initiated changes in agronomic practices, eventually resulting in production advances.

Nutritional adaptation is widespread in nature and there are distinct genotypic differences for response patterns to nutritional elements, and toxicities as well. At an application level up to 50 kg N/ha, sorghum hybrids and some improved varieties have returned 15-28 kg of grain per kg of nitrogen against 6-8 kg for traditional locals. Further, in hundreds of nitrogen response trials conducted all over India during several years, we never observed crossing over of response curves to indicate that certain genotypes (including locals) were higher yielding at lower levels of nitrogen application, or, conversely, where any hybrids were yielding less than others at lower levels but outyielded them beyond certain level of fertilizer dosage.

Similarly, several studies on genotype responses to population levels in all India trials established the superiority of altered genotypes for community performance compared with the improved local cultivars. Population levels of 1,80,000-2,00,000 plants per hectare were optimal at the field level.

The response of altered genotypes to fertilizer and population levels are more



spectacular and coupled with their lower susceptibility to climatic variables, notably rainfall, their adoption rate is on the increase, although the level of fertilizer used on commercial sorghum fields is still low.

Considering all agronomic inputs, including the use of fertilizers, pesticides, etc., it has been stated frequently that the performance of high-yielding hybrids and varieties remains satisfactory only under optimal inputs (including irrigation water) and management, and that their yields will not be satisfactory in the absence or under lower levels of such inputs and management. Consequently, 'high yield agriculture' is associated with 'high input agriculture' and it is questionable whether this is applicable to the small farmers in developing countries. This aspect has been examined in multilocation experiments conducted over years. The top ranking hybrids and varieties maintained their relative ranks under both types of input-management levels<sup>4,5</sup>.

These and various other studies indicate that agriculture based on altered genotypes is not incompatible with lower input levels, and the actual level and use of inputs gets into the realm of availability, supply, credit and related matters rather than technology-imposed limitations. While the yield levels may vary depending on input use, altered genotypes did confer greater levels of stability and productivity. In some years and areas, the differences have been of the order of economic yields against total failure.

### **Sorghum-based Cropping Systems**

One has to first realize that the component cultivars of the traditional intercropping systems are themselves the

products of climate vulnerable subsistence agriculture. But for the spread of risk cover over species, they are essentially replacement systems characterized by low yields. Unless the components themselves undergo radical alteration, the system will not alter. We<sup>10</sup> demonstrated that sole crop stability and productivity is a prerequisite for productive intercropping systems. Further, based on studies involving inter- and intra-species competition, genotype  $\times$  density interactions and alternate planting patterns, we examined the design and practice of stable, productive and profitable intercropping systems. Summarizing data from several all India trials, we furnished evidence of such systems which involved sorghum as the principal crop with 90-95% of the sole crop yield and pigeonpea, soybean and groundnut as intercrops. New and more remunerative crops like onion, garlic, etc., are now being experimented to enhance returns. Traditional intercropping systems which have given place to sole crops of hybrid sorghum have now been oriented towards more profitable intercropping.

While such intercropping systems are advantageous in areas of relatively low rainfall, multiple cropping is more profitable in high rainfall areas with moisture retentive soils. A vast portion of the black soil belt of the Deccan and central Indian plateaus with 800 mm of annual rainfall which sustained 5-6 months' crops of traditional sorghums can now take as assured crop of short-season hybrid in all years and a following crop of safflower or chickpea in normal and above normal years of rainfall. We<sup>10</sup> demonstrated that the present shortages of grain legumes and edible oilseeds could be met by the practice of sorghum-based

inter- and sequence-cropping on existing sorghum acreages<sup>10-12</sup>.

That productivity per year in the tropics will probably be achieved through attempts to maximize the number of crops rather than yield of each crop is relevant for areas where traditionally long season sorghums have been under cultivation in India and Africa. Emphasis on the manipulation of the cropping system based on modified cultivars will be more fruitful, as demonstrated in India, than attempts to breed improved cultivars comparable to late locals in maturity.

### Impact on Sorghum Production

The impact of the altered hybrids, which are of much shorter duration, compared to the traditional sorghums, has been preceptible on overall sorghum production and productivity in India. In spite of the fact that the hybrid coverage has been confined to the rainy season in some districts, primarily in the states of Maharashtra and Karnataka, this limited spread itself had an overall impact on sorghum production (Table 1). The impression that the green revolution is confined to only irrigated crops like wheat and rice is erroneous and sorghum is an example that it could encompass rainfed areas as well.

**Table 1—Compound Growth Rates of Area, Agricultural Production and Yield during 1967-68 to 1978-79 on All-India Basis**

	(Values are in %)		
	Area	Production	Yield
Sorghum	1.49	2.07	3.62
Wheat	3.16	6.02	2.76
Rice	0.82	2.64	1.80
Pearl millet	-1.26	0.28	1.53
Maize	0.05	-0.04	-0.07

### Basic Researches

A series of papers<sup>2,4,6</sup> have been published on the genetics of tropical × temperate crosses involving studies on heterosis, combining ability, adaptation and stability, selection criteria, methods and advance, insect and disease resistance furnishing the basis for sorghum improvement. The discovery of apomixis in sorghum became the basis of breeding permanent hybrids<sup>13,14</sup>. Studies on nutritional quality of sorghum<sup>15</sup>, analysis of diverse cytoplasmic systems<sup>16</sup> and the design and development of optimum plant types<sup>17</sup> offer avenues for future improvement of sorghums. Over 250 research papers concerning improvement of sorghum, pigeonpea, cotton, groundnut, castor, etc. have been published.

### Future Approaches

Rabi sorghum improvement needs special attention. Efforts to make jowar proteins more nutritious by incorporating higher levels of lysine should be pursued. As jowar production increases, the jowar grain could find a place in the feedgrain industry. Jowar has also an export potential as a feedgrain. Sorghum straw, if suitably enriched with sugars, could become an important non-conventional and renewable energy source. Grain sorghums have also industrial potential which needs to be exploited. The All India Sorghum Improvement Project should gear itself to meet these future challenges.

### Selected Publications

1. Swaminathan M S, Dhawan N L, Murthy B R & Rao N G P, Genetic improvement of crop plants initiates an era of vanishing barriers, *ICAR Year Book*, 1970, 33-146.
2. *Sorghum in seventies*, edited by N G P Rao & L R House (Oxford and IBH, New Delhi) 1972, pp 636.



3. Rao N G P, Five years of sorghum breeding, S S Bhatnagar Memorial Award Lecture, *J scient ind Res*, **31** (1972) 498-509.
4. Rao N G P, Genotype alteration: The basis of agricultural transformation in tropical drylands, *Presidential Address to the Indian Society of Genetics and Plant Breeding*, *Indian J Genet*, **41** (1981) 437-49.
5. Rao N G P, Transforming traditional sorghums in India, in *Sorghum in Eighties*, *Proc Int Symp on Sorghum*, ICRISAT, Hyderabad, 1982, 39-59.
6. Rao N G P & Rana B S, Selection in temperate tropical crosses of sorghum, *Proc Int Symp on Sorghum in Eighties*, ICRISAT, Hyderabad, 1982, 403-19.
7. Barih B C, Binswanger H P, Rana B S & Rao N G P, The use of risk aversion in plant breeding: concept and application, *Euphytica*, **30** (1982) 451-58.
8. Rao N G P, Vidyabhushanam R V, Rana B S, Jayamohan Rao V & Vasudeva Rao M J, *Breeding sorghums for diseases resistance in India*, *Sorghum diseases: A world review* (ICRISAT, Hyderabad) 1978, 430-33.
9. Rana B S, Singh B U & Rao N G P, Breeding for shootfly and stem borer resistance to sorghum, *Proc Int Symp on Sorghum Insect Pests*, Texas, USA, 1985, 347-60.
10. Rao N G P & Rana B S, Sorghum based cropping systems to meet shortages of pulses and oilseeds in India, *Curr Sci*, **33** (1980) 155-62.
11. Rao N G P, Rana B S & Tarhalkar P P, Stability, productivity and profitability of some intercropping systems in dryland agriculture, *Proc Int Symp on Intercropping*, ICRISAT, Hyderabad, 1979, 55-140.
12. Rao N G P, Sorghum production in relation to cropping systems, *Proc Int Symp on Sorghum Insect Pests*, Texas, USA, 1985, 3-11.
13. Rao N G P & Narayana L L, Apromixis in grain sorghums, *Indian J Genet*, **28** (1968) 121-27.
14. Murthy U R, Kirti P B, Bharati M & Rao N G P, The problems of apromixis and its prospects in the eighties, *Proc Int Symp on Sorghum in Eighties*, 1982, 361-72.
15. Jayamohan Rao V, Reddy B B, Rana B S & Rao N G P, Breeding for superior quality storage proteins in grain sorghum, *Indian J Genet*, **43** (1983) 395-403.
16. Rao N G P, Tripathi D P & Rana B S, Genetic analysis of cytoplasmic systems in sorghum, *Indian J Genet*, **44** (1984) 480-96.
17. Rana B S, Barah B C, Binswanger H P & Rao N G P, Breeding optimum plant types in sorghum, *Indian J Genet*, **44** (1984) 385-98.

## Satya P Raychaudhuri\*

During 1927-1934, as a research student under Prof. J N Mukherjee, University of Calcutta, Raychaudhuri carried out electrochemical studies on soil colloids and clays; this work involved electrometric and conductometric titrations. It was shown that soil clays behave as an acid, in some respects stronger than hydrochloric acid. His work on activated charcoal revealed its usefulness as an effective adsorbing agent for various cations.

While at Rothamsted Experimental Station, England (1934-36), Raychaudhuri carried out intensive work on the variations in physico-chemical properties of the neighbouring tropical soils of Nyasaland Protectorate, British Central Africa. On his return to India, he initiated research on red and lateritic soils of India; his results have been widely quoted by foreign authorities in their research papers, bulletins and textbooks. His studies on saline and alkali soils provided the base of work on these soils in the country. On the basis of the existing knowledge, the saline and alkali soils were classified into different categories. As Soil Survey Officer under ICAR Project, Raychaudhuri reviewed the progress of soil survey work in the country; this led to the preparation of a Soil Map of India and initiation of all India soil survey work on a uniform basis in the country. A handbook on soil survey was prepared for providing a uniform pattern for soil and land use

survey work in different parts of India. As Head of the Division of Soil Survey and Agricultural Chemistry at the Indian Agricultural Research Institute and Incharge of Indo-US Project on Soil Fertility and Fertiliser Use, Raychaudhuri initiated research and development work on (1) soil testing for efficient fertilizer use, (2) balanced use of fertilizers, including organic manures, for the important crops in different parts of the country, and (3) keeping qualities of various chemical fertilizers under different storage conditions. The trials conducted established that contrary to the then prevailing notions, nitrophosphate, which can be produced without the use of sulphur or sulphuric acid, is a suitable chemical fertiliser under Indian conditions.

Under the guidance of Raychaudhuri, while he was Head of the Division of Soil Science and Agricultural Chemistry, Indian Agricultural Research Institute, a comprehensive book entitled "*Soils of India*" was published in 1963 by ICAR, with him as the chief author. After his retirement in 1961, Raychaudhuri worked till 1969 as Senior Specialist (Land Resources), Planning Commission. During this period he published (1967), through the National Book Trust of India, a book entitled "*Land and Soil*" containing maps of soils, ravines, saline and alkali soils and micronutrient status in different parts of the country. This book, which is meant for

---

Formerly, Head, Division of Soil Science and Agricultural Chemistry, Indian Agricultural Statistics Research Institute, New Delhi-110012; and Senior Specialist, Land Resources, Planning Commission, Government of India.

\*Science deceased.



use by progressive farmers and extension workers, passed through the second edition in 1969 and the third edition in 1981. The book has been got translated by the National Book Trust of India into all the important languages of India.

Raychaudhuri has carried out and guided intensive work on soil and water conservation and published a bulletin along with Prof. Hans Jenny of USA entitled, "*Effect of climate and cultivation on nitrogen and organic matter resources in Indian soils*". First published by ICAR in 1960, the bulletin was reprinted in 1967. It has become a standard reference source both nationally and internationally.

Raychaudhuri attended on invitation international soil science conferences at Rome and Tashkent (USSR) in 1961, 1962 and 1963 under FAO-UNESCO programme as representative of South East Asia for the preparation of a soil map of the world, which has since been published. He presented a paper on invitation from UNESCO on the sodic soils of India at an international conference in 1964 at Budapest. He also published a soil map of West Bengal showing the occurrence of these soils. He also attended two international conferences on chemicalisation of plant production under invitation from the Institute of Tropical Agriculture, Karl Marx University, Leipzig (D.D.R.) in 1977, where he presented a paper on "Integrated nutrient supply". At the fifth international Summer Seminar, Institute of Tropical Agriculture, Karl Marx University, Leipzig (D.D.R.) on 'Agrarian reform and rural development in Asia, Africa and Latin America 1980', he presented a paper on the "Small and marginal farmers of India".

The publications of Raychaudhuri include 96 original research papers, 150 survey, development and review reports, 25 books/chapters in books and bulletins, and 13 miscellaneous reports. He guided the postgraduate research work of 55 students.

Raychaudhuri is currently engaged in writing monographs on agriculture in ancient India and in mediaeval India, under schemes financed by the Indian Council of Agricultural Research and the Indian National Science Academy respectively. He is the technical editor and coordinator for the project on the revision of the book 'Soils of India' published by ICAR in 1963. He proposes to write a book on Imperial agriculture in India (1765-1947).

Satyaprasad Raychaudhuri is an elected member of the World Academy of Art and Science and an Honorary member of the USSR Society of Soil Scientists.

### Selected Publications

1. Raychaudhuri S P, Studies on the physico-chemical properties of associated black and red soils of Nyasaland Protectorate, British Central Africa, *Indian J agric Sci*, **11** (1941) 100-9.
2. Raychaudhuri S P & Subbiah B V, Phosphate manuring of legumes and its role in the building up of the fertility of Indian soils, *Proc Session 7, Int Nat Cong Soil Sci*, 1952, 253-58.
3. Raychaudhuri S P & Mathur L M, A basic land resource map of India, *Bull natn Inst Sci India*, (3) (1954) 111-14.
4. Shome K B & Raychaudhuri S P, Rating of soils of India, *Proc natn Inst Sci India*, **26A** (Suppl 1) (1960) 260-89.
5. Raychaudhuri S P, The quality of ground water for irrigation in arid and semi-arid regions, *Proc Symposium on ground-water, studies in arid and semi-arid regions*, University of Roorkee, 27-30 October 1968 (Department of Geology and Geophysics University of Roorkee) 1969, 339-46.
6. Raychaudhuri S P, Fertilizer demonstrations under Shriram Khad Programme on Soil Test

- Basis, *Proc Int Symp on Soil Fert Evaln*, Vol 1, New Delhi, 1971, 1093-1102.
7. Ghosh K & Raychaudhuri S P, Distribution of clay minerals in Indian soils, in *Mineralogy of Soil Clays and Clay Minerals*, Indian Soc of Soil Sci (JN Mukherjee Sp Issue), Bull No. 9, 1974, 143-64.
  8. Raychaudhuri S P, Agricultural practices in ancient India, *ICAR Rev Ser*, (4) (1953) 1-48.
  9. Blume J M & Raychaudhuri S P, Soil fertility and how to maintain it, *ICAR Farm Bull* No. 30, 1957, 1-18.
  10. Raychaudhuri S P & Subbiah B V, Soil testing kit, *ICAR Res Ser* No. 14, 1958, 1-36.
  11. Jenny Hans & Raychaudhuri S P, *Effect of climate and cultivation on nitrogen and organic matter reserves in Indian soils* (Indian Council of Agricultural Research) 1960, 1-127 (reprinted, December 1967, pp 127).
  12. Raychaudhuri S P, Agarwal R R, Datta Biswas N R, Gupta S P & Thomas P K, *Soils of India* (Indian Council of Agricultural Research) 1963, pp 496.
  13. Raychaudhuri S P & Datta N P, Phosphorus and potassium status of Indian soils, *ICAR Rev Ser*, Bull No 36, 1964, pp 43.
  14. Raychaudhuri S P, *Land resources of India*, Vol I, *Indian soils—Their classification, occurrence and properties* (Committee on Natural Resources, Planning Commission, Government of India) 1964, pp 89.
  15. Raychaudhuri S P, *Land and soil* (National Book Trust of India, New Delhi, 1966, 1-155; revised 1969, pp 190; re-revised 1981, pp 188.



## Syama P Raychaudhuri

Raychaudhuri started his career as a mycologist and plant pathologist in 1939 at the Indian Agricultural Research Institute, New Delhi, where he worked on a new fungus causing collar rot of pigeon pea under the guidance of Dr G Watts Padwick, the then Imperial Mycologist. Later, at Dacca, he worked on false smut, sclerotial diseases and helminthosporium of rice. He found out the mode of floral infection of false smut, which could not be done earlier in association with later Prof. P Maheshwari. He discovered powdery mildew of cotton while in Dacca. He also worked in association with Dr L O Kunkel in U.S.A., Dr B N Uppal in Poona and Prof. J F Dastur in Delhi.

Later, important work on zinc deficiency of guava which was a baffling problem in Rajasthan was done by him; this problem was overcome by spraying zinc sulphate on the diseased trees.

He was the first scientist in India to purify and crystallize a plant virus causing mosaic of sunhemp. Later, he did a lot of work on the virus diseases of various crops in Delhi and Kalimpong areas. He developed resistant varieties and chemical control methods for curing diseases of plants, particularly of cereal crops and citrus. His basic findings on tissue and meristem culture in relation to plant viruses and plant virus inactivation have been quoted in standard textbooks.

He and his colleagues worked on coconut root wilt as well as mango malformation, the etiology of which was not clearly understood earlier. He found out that citrus dieback in Northern India is caused by 'virus-fungus', fungus-mycoplasma or a complex of all the three pathogens. Greening mycoplasma was cultured for the first time in his laboratory; he proved that the cultured mycoplasma could infect the vectors of the disease-causing greening as well as the healthy citrus plants under laboratory conditions, in pot culture house as well as in some cases in the field. Signs of recovery were found in greening disease of citrus by applying antibiotics. He also made interesting findings in market pathology. He initiated research on virus diseases of cereals, maize, millets and cardamom. In recent years, the most exciting result from his work has been on sandal spike, which is an age old problem and which takes a heavy toll. It was found out by him and his group that it occurs due to mycoplasma.

The major contributions of Raychaudhuri are summarized below.

### (1) Citrus Dieback

The nature of citrus dieback was established as a result of the work done by him and his team of workers which has proved that the viruses, tristeza and exocortis and mycoplasma cause citrus

---

Formerly, Head, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012.

greening and 5 of the major fungi jointly are responsible for causing citrus dieback, specially in Northern India. Due to infection with the virus or greening disease, the plant becomes weak after the leaf fall fungal spores enter and cause woodrotting. Citrus greening has been successfully checked by heat treatment and chemotherapy. Attempts were also made to raise healthy clones of citrus free from exocortis virus which is so far not known to have any vector, so that these healthy clones can be grown outside and healthy orchards may be established by using budwood from these viroid-free materials. Such an attempt is being made for the first time in the country and mollicata causing citrus greening has been successfully cultured. Koch's postulate has been proved for the first time. A new technique based on fluorescent microscopy has been employed in detecting greening mycoplasma in the citrus greening diseased tissues.

## **(2) Mango Malformation**

The etiology of this condition was not fully understood; it was known that mites cause the disease in mango growing areas, specially in north and eastern parts of India. It has been proved by Raychaudhuri and his coworkers that mites carry spores of *Fusarium moniliforme* that enter into the tissues of mango plants; *Fusarium* produces a malformation which is more or less like pin-cushion disease of cocoa in South America. Attempts are being made to control the disease by using several pesticides; partial success has already been achieved in both greenhouse and orchards.

## **(3) Sandal Spike**

This disease, an age-old problem, was known to be due to virus which, however,

was never isolated. Recently, Raychaudhuri, in collaboration with the Forest Research Laboratory of Bangalore, has been able to prove that it is due to mycoplasma; using benlate (a systemic pesticide) along with antibiotics like terramycin and ledermycin, excellent recovery of the disease symptoms has been observed in both greenhouse and in the forests of Bangalore and Mysore. The technique used involves stem girdling; retention of the chemical is being studied now.

## **(4) Cereal Viruses**

Raychaudhuri is responsible for initiating virus work, specially in respect of cereals like rice, wheat, barley, etc. He discovered the presence of tungro virus and yellow dwarf disease of rice; he also established their vector in India and mosaic streak of wheat and cereal yellow dwarf as well as streak disease of bajra which has seven hosts, including wheat. In addition to finding sources of resistance, it has been possible for him to find out preventive measures including seed treatments of rice with systemic pesticides so that the leaf-hopper vectors do not feed on rice plants in seedbed or in the field for developing tungro and yellow dwarf diseases. He has also done fundamental work on electron microscopy of these viruses.

## **(5) Market Pathology and Storage Diseases**

Raychaudhuri initiated for the first time in India studies on market pathology and storage problems, including in transit. As a result of his work, it has been found out that Diplodia rot of mango and the Alternaria rot of tomato can be controlled during transit and these important fruits and vegetables can be protected for at least 2-3 weeks, so that they reach their



destination from one part of the country to the other without getting spoiled. Post-harvest treatment with Aureofungin has been very effective in checking the disease during this period, so that their market value is not reduced. Work done in collaboration with the Defence Science Laboratory, Kanpur, revealed that by using VFI (vaporised fungal inhibiting) tablets deterioration of stored groundnut seed through the formation of aflatoxins by *Aspergillus flavus* can be checked.

#### **(6) Inhibition of Plant Viruses and Tissue Culture Studies**

Extensive studies were done by Raychaudhuri on viral inhibition by chemotherapy. Some of the findings are of great interest from both fundamental and applied points of view. The studies have been made not only with glasshouse test plants or in the field but also in tissues and meristem culture to produce virus-free healthy clones of plants. For the first time gamma radiation has been used in combination with various chemicals for eliminating virus from infected cells.

#### **(7) Basic Studies on Plant Mycoplasma**

During the last 15 years, Raychaudhuri has been responsible for establishing a strong school of research on 'Plant mycoplasma diseases' in the country engaged in fundamental work on culturing techniques using modern methods like electron micrography and fluorescent microscopy.

Raychaudhuri is a Fellow of the Indian National Science Academy; National Academy of Sciences, India; Linnean Society of London; and Indian Phytopathological Society and is the only fellow of the American Phytopathological

Society from India. He is past President of Section of Agricultural Sciences of Indian Science Congress; Indian Phytopathological Society; first Honorary Treasurer of the International Society for Plant Pathology; Executive Councillor of the International Society of Citriculture from India. He has been President and Chief Editor of *Indian Phytopathology* and has been Chairman of Organizing Committee of two international symposia held in New Delhi in 1966 and 1971 and also Convenor jointly with Dr Paul Neergaard of the 11th Seed Pathology Workshop held for the first time in Asia in New Delhi in 1967. Raychaudhuri is President of Indian Virological Society, Indian Mycological Society and Indian Society of Citriculture, and past President of the Society for Advancement of Botany and the Indian member of Advisory Board of *Plant Pathology Treatise* (5 volumes) being brought out by the Academic Press (USA). He is Honorary Fellow of the Indian Phytopathological Society and Indian Mycological Society.

Raychaudhuri has more than 300 scientific publications and has written invitational articles in many reputed books and journals and has written and edited 12 books.

In recognition of his contribution on sandal spike diseases (Mycoplasma) and citrus greening he has been elected as Chairman (1977-86) of Working Party on Mycoplasma Diseases by IUFRO (International Union of Forestry Research Organisations) and Chairman of Citrus Dieback Committee of International Society of Plant Pathology (1978-83).

Raychoudhuri won the Rafi Ahmed Kidwai Memorial Prize in 1966-67 and was a Fulbright Scholar (1950-52).

## Selected Publications

1. Raychaudhuri S P, A disease of pigeon pea (*Cajanus cajan* L) (Mill sp) caused by *Diplodia cajani* sp nov, *Indian J agric Sci*, **12** (1942) 387-471.
2. Raychaudhuri S P, Mode of infection of rice by *Ustilaginoidea virens* (Oke) Tak, *J Indian bot Soc*, **25** (1946) 145-50.
3. Raychaudhuri S P, A note on the mosaic virus in sunnhemp (*Crotalaria juncea* Linn) and its crystallization, *Curr Sci*, **16** (1947) 26-28.
4. Raychaudhuri S P, *Oidiopsis gossypii* (Wakef) Raychaudhuri F nov on cotton, *Trans Br mycol Soc*, **32** (1949) 288-90.
5. Raychaudhuri S P, Studies on internal browning of tomato, *Phytopathology*, **42** (1952) 591-95.
6. Raychaudhuri S P, Plant viruses in tissue culture, *Adv Virus Res*, **12** (1966) 175-206.
7. Raychaudhuri S P, Plant viruses in plant and insect tissue culture, in *Tissue culture*, edited by C V Ramakrishnan (Dr W Junk Publishers, The Hague) 1967, 244-50.
8. Raychaudhuri S P, Development of mycological and plant pathological research, education and extension work in India, *Rev appl Mycol*, **46**(11) (1967) 577-83.
9. Raychaudhuri S P & Ganguly B, A mosaic streak of wheat, *Phytopath Z*, **62** (1968) 61-65.
10. Raychaudhuri S P, Intensification of seed pathology work in India and need for proper quarantine measures, *Proc Int Seed Test Ass*, **35** (1970) 69-76.
11. Raychaudhuri S P, Verma J P, Nariani T K & Sen B, The history of plant pathology in India, *Ann Rev Phytopath*, **10** (1972) 21-36.
12. Raychaudhuri S P, Seed pathology in relation to plant quarantine and seed industry in India, *Indian Phytopath*, **26** (1973) 185-96.
13. Raychaudhuri S P, Mycoplasma diseases of plants, *Acta Bot Indica*, **2**(1) (1974) 13-16.
14. Raychaudhuri S P & Verma J P, Therapy by heat, radiation and meristem culture, *Plant Disease*, Vol I (Acad Press, Inc) 1977, 177-89.
15. Raychaudhuri S P & Varma Anupam, Sandal spike, *Rev Pl Path*, **59**(3) (1980) 99-107.
16. *Plant disease problems, First International Symposium*, edited by S P Raychaudhuri, G Malchers, R Prasada, G Morel, M J Thirumalachar, G Rangaswami, T S Sadasivan, B L Renfro, M M Payak, Kishan Singh, V V Chenulu, V P Bhide, C S Holton & L M Joshi (Indian Phytopathological Society) 1970, pp 915.
17. *Current trends in plant pathology*, edited by S P Raychaudhuri and J P Verma, 1974, pp 341.
18. Raychaudhuri S P, A manual of virus diseases of tropical plants (MacMillan Co of India Ltd) 1977, pp 300.
19. Raychaudhuri S P & Nariani T K, Virus and mycoplasma diseases of plants in India (Oxford & IBH Publishers, New Delhi) 1977, pp 102.
20. *Mycoplasma diseases of trees*, edited by S P Raychaudhuri (Associated Publishing Co, New Delhi) 1979, pp 78.
21. *Environmental pollution and toxicology*, edited by S P Raychaudhuri and D C Gupta (Today & Tomorrow's Printers and Publishers, New Delhi) 1980, pp 341.
22. Maramorosch Karl & Raychaudhuri S P, *Mycoplasma diseases of trees and shrubs* (Academic Press Inc, New York) 1981, pp 362.
23. *Problems of citrus diseases in India*, edited by S P Raychaudhuri and Y S Ahlawat (Surabi Printers & Publishers, New Delhi) 1982, pp 134.
24. *Current trends in plant virology*, edited by B P Singh and S P Raychaudhuri (Today & Tomorrow's Printers and Publishers, New Delhi) 1982, pp 223.
25. *Citrus production—Challenging problems*, edited by S P Raychaudhuri & O S Rakesh (Indian Society of Citriculture/Surabi Printers & Publishers, New Delhi) 1985, pp 168.



## D P Sadhu

Sadhu did his PhD from the University of Missouri, USA. The main areas of his work were thyroid physiology, with special reference to goitrogenesis, vitamin A, with reference to hypervitaminosis A and stereothermal regulation in animals.

One of the earliest investigations was on the correlation between acetylcholine and vitamins in the body muscle. The results obtained were presented at a biochemistry seminar at the University of Missouri in 1948.

Another line of investigation related to the nutritional significance of lactose. The lactose content of mother's milk was correlated with the development of the brain. The aim of another investigation was to study the mechanism of specific dynamic action of nutrients, with special reference to proteins. The heat increment of feeding was correlated with the level of oxidative deamination in the liver and it could be reduced by the process of transamination. In ruminant animals, the rumen is anaerobic and there is a lot of transamination in the rumen liquor. Vitamins and hormones were found to be correlated with the heat increment of feeding. Thiouracil and basal metabolism were correlated with specific dynamic action. Pyridoxine and ketonic acids were also correlated with a specific dynamic action.

Vitamin A level was correlated with thyroid size. The physiological mechanism of experimental goitrogenesis was established. Vitamin A, iodine and thyrotropic contents of anterior pituitary were correlated. Hypervitaminosis A and reticulo-endothelial system blockage were found to affect the thyroid. Hypervitaminosis A affected the distribution of body iodine also. The formation of thyroxine and its precursors was correlated with iodoproteins and iodopeptones.

Some of the other aspects investigated were as follows:

(1) Mechanism of formation of biochemical lesions by *l*-tyrosine.

(2) Role of ascorbic acid in transglycosidation.

(3) Influence of lactose on the partition of sphingosine between galactose and choline phosphate.

(4) Physiological significance of lactose in pigeons.

(5) Influence of lactose on transglycosidation of sphingosine base in the rat brain.

(6) Role of thyroid in experimentally induced cholesterologenesis.

(7) Cholesterol synthesis in pantothenic acid deficiency condition.

(8) Metabolism of acetic acid in kidney and liver slices of hypothyroid rats.

(9) Mechanism of cholesterol synthesis in nephrosis.

(10) Metabolic depression caused by arsenite and melonate and cholesterologogenesis.

(11) Histopathology of nephrotic kidney.

(12) Tissue respiration of slices.

(13) Response of blood sugar and serum cholesterol in rabbit exposed to high ambient temperature.

(14) Neural basis of thermoregulation in guineapigs.

Based on the study of the evolution of temperature regulation in animals, a new classification of animals into pleothermal, stenothermal, etc. was established.



## S V S Shastry

Commencing in 1950, Shastry has devoted 25 years to active scientific research and 10 years to research management. With the exception of the PhD thesis on *Melilotus* (sweet clover) and guiding of PhD students on the cyogenetics of *Rosa* (roses), his research has been exclusively on the genus *Oryza* (rice) covering various aspects—physiology, agronomy, breeding, genetics, cytogenetics, taxonomy and finally crop improvement and development. As a research manager and adviser, Shastry headed the multidisciplinary research programme at the International Institute for Tropical Agriculture, Ibadan, Nigeria and functioned as the Executive Secretary of the International Rice Commission at the headquarters of the Food & Agriculture Organization in Rome. His major contributions are as follows:

(1) Established a generality in the quantum of photoperiodic response of the rice varieties.

(2) Demonstrated that high sterility in the  $F_1$  hybrids of *japonica* and *indica* rices is no impediment to the breeding of fertilizer-responsive plant types.

(3) Established that chromosome structural differentiation and isolating mechanisms progress independently of each other in the process of speciation in the genus *Melilotus*.

(4) Developed the technique of pachytene analysis in *Melilotus* and utilized it in interpreting chromosome structural differentiation in speciation.

(5) Developed the technique of pachytene analysis in the genus *Oryza* and utilized it in the study of evolution of karyotypes.

(6) Interpreted the genomic differentiation between *Oryza sativa* and *O. officinalis* as being due to desynapsis.

(7) Established the “timing imbalance” in the meiosis of the  $F_1$  hybrid *O. sativa*  $\times$  *O. australiensis*.

(8) Demonstrated conclusively that the sterility in the intervarietal rice hybrids is chromosome structural rather than genic, as supposed earlier.

(9) Did cytogenetical analysis of triploid *Oryza* hybrid and drew inferences on karyology based on the shape of multivalents.

(10) Proposed a revised taxonomic classification of the genus *Oryza*.

(11) Enumerated some key characters hitherto neglected in the taxonomy of *Oryza*.

(12) Named the species, *O. nivara*, and gave correct designation of *O. rufipogon* and *Sclerophyllum coarctatum*.

---

Formerly, Director of Research, International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria; Residence : B-4, Samrat Complex, Saifabad, Hyderabad-500004.

(13) Enquired into the putative ancestor of cultivated rice.

(14) Achieved the induction of mutations in the different species of *Oryza*; developed short culm mutants in the variety NP 130.

(15) Determined the pattern of internodal contraction in various semidwarf mutants in rice.

(16) Developed semi-dwarf high yielding varieties of rice—Jaya, Phalguna, Cauvery, Sona, etc. Jaya holds till today an eminent place in farmers' patronage.

(17) Studied the genetics of resistance to bacterial leaf blight, tungro virus, stem borer and gall midge in rice.

(18) Explored the primitive cultivars of rice from North East Indian hills, assessing the germplasm and identifying new sources of resistance to insects and diseases and new sources of good plant type.

(19) Maximized the effectiveness of fertilizer in the management of rice.

(20) Established the stability in performance of semi-dwarf varieties over a wide range of environmental conditions and management.

### Future Plans for Research

(1) Development of good plant type and high yielding varieties of rice capable of adapting to marginal rice growing conditions, such as upland rainfed situations.

(2) Land management for upland rice in the highly erodable soils.

(3) Making use of the diverse sources of dwarfism and redesigning of a plant type

even better than what is now available in the semi-dwarf varieties from Taiwan.

### Selected Publications

1. Ghose R L M & Shastry S V S, Response of rice varieties to short-day treatment, *Euphytica*, **3** (1954) 221-28.
2. Shastry S V S, Smith W K & Cooper D C, Chromosome differentiation in several species of *Melilotus*, *Am J Bot*, **47** (1960) 613-21.
3. Shastry S V S & Misra R N, Pachytene analysis in *Oryza*. II. Sterility in *Japonica-indica* hybrids, *Chromosoma*, **12**(2) (1961) 248-71.
4. Shastry S V S, Sharma S D & Ranga Rao D R, Pachytene analysis in *Oryza*. III. Meiosis in an intersectional hybrid, *O. sativa* × *O. officinalis*, *Nucleus*, **4** (1961) 67-80.
5. Shastry S V S & Ranga Rao D R, Timing balance in the meiosis of the  $F_1$  hybrid *Oryza sativa* × *O. australiensis*, *Genet Res*, **2**(3) (1961) 373-83.
6. Shastry S V S, Chromosome structural differentiation, isolating mechanisms and speciation in *Oryza*. Rice Genetics and Cytogenetics, *Proc Symp Int Rice Research Institute*, Los Banos, February 1963 (Elsevier Publishing Co, Amsterdam) 1964, 111-17.
7. Shastry S V S, Is sterility genic in *Japonica indica* rice hybrids? Rice Genetics and Cytogenetics, *Proc Symp Int Rice Research Institute*, Los Banos, February 1963 (Elsevier Publishing Co, Amsterdam) 1964, 154-57.
8. Shastry S V S & Misra R N, Frequency and types of multivalents in a triploid *Oryza* hybrid and their cytogenetic significance, *Nucleus*, **7**(1/2) (1964) 93-104.
9. Sharma S D & Shastry S V S, Neglected characters in taxonomy of genus *Oryza* L, *Curr Sci*, **33**(10) (1964) 316-17.
10. Sharma S D & Shastry S V S, Taxonomic studies in genus *Oryza*. III. *O. rufipogon* Griff *sensu stricto* and *O. nivara* Sharma et Shastry nom nov, *Indian J Genet*, **25**(2) (1965) 157-65.
11. Shastry S V S, X-ray induced mutations in cultivated rice, NP 130, *Curr Sci*, **34**(2) (1965) 55-56.
12. Shastry S V S, Taxonomic studies in genus *Oryza* V *Sclerophyllum coarctatum* (Roxb) Griff, *Bull bot Surv India*, **8** (1966) 42-44.
13. Misra R N & Shastry S V S, Desynapsis and intragenomic differentiation in cultivated species of *Oryza*, *Cytologia*, **34**(1) (1969) 1-5.



14. Shastry S V S, New high yielding varieties of rice—Jaya and Padma, *Indian Fmg*, **18**(11) (1969) 5-13.
15. Shastry S V S, Sharma S D, John V T & Krishnaiah K, New sources of resistance to pests and diseases in the Assam rice collections, *IRC Newslett*, **20** (1971) 1-16.
16. Jayaraj D, Seshu D V & Shastry S V S, Genetics of resistance to bacterial leaf blight of rice, *Indian J Genet*, **32**(1) (1972) 77-89.
17. Shastry S V S, Toward a rice revolution, *Indian Fmg*, **22**(5) (1972) 95-97, 99-112.
18. Shastry S V S & Sharma S D, Rice, in *Evolutionary studies in world crops : Diversity and change in the Indian sub-continent*, edited by J Hutchinson (Cambridge University Press, London) 1973, 55-63.
19. Sharma S D & Shastry S V S, Evolution in genus *Oryza*, in *Advancing Frontiers in Cytogenetics* (Hindustan Publishing Corporation, Delhi) 1973, 5-20.
20. Seshu D V, John V T & Shastry S V S, Incorporation of resistance to tungro virus in dwarf rice varieties, *Proc II SABRAO Congress*, New Delhi, 22-28 February 1973, *Indian J Genet*, **34A** (1973) 450-57.
21. Seshu D V, Prasada Rao U, Srinivasan T E & Shastry S V S, New sources of dwarfism in the Assam rice collections, *Indian J Genet*, **34**(3) (1974) 390-94.
22. Shastry S V S, Fertilizer—A component, not a substitute for good management, *Indian Fmg*, **23**(11) (1974) 15-17.
23. Dutt K V L N, Seshu D V & Shastry S V S, Genetics of resistance to rice stem borer, *Int Rice Res Newslett*, **3**(6) (1978) 11.

## N S Subba Rao

### Rhizosphere Biology

A significant diagnostic measure for the incipient detection of wilt symptoms in cotton infected with *Fusarium vasinfectum* even before the onset of the disease was discovered. It involved selective fluorescence of plant parts of infected seedlings under UV light. Transverse sections of stem from infected seedlings showed fluorescence of phloem vessels followed by xylem as the infection spreads.

Simple and effective methods to collect and analyze the root exudates of crop plants were devised. Various amino acids, sugars and organic acids liberated from the roots of different crop plants into the rhizosphere were identified through paper chromatographic analysis. The pathway of nutrients from the soil into plants was found to be mediated by root surface fungi. For the first time, the transport of labelled phosphate and carbon from soil to plants and vice versa was shown with the help of labelled nutrients; this represented a major advance in our knowledge of rhizosphere biology.

### Biological Nitrogen Fixation in Legumes

The association of fungi in the cortical cells of root nodules and the part played by such fungi in phosphate solubilization was demonstrated. Several species of soil

fungi which accelerate the growth of rhizobia were distinguished from those which inhibit growth. This information provided a clue to the success or failure of rhizobia to establish in the rhizosphere.

Evidence was provided from studies with lucerne and *R. meliloti* to show that the legume is more sensitive to different salts than the *Rhizobium* partner and that nodulation failure in saline soil is due to the inability of *Rhizobium* to infect plant roots in spite of high populations in the rhizosphere even under extreme salinity conditions.

*Rhizobium* species reacted differently to the presence of the herbicides amitrole, paraquat, 2,4-D, 2,4-DB and MCPB. The responses ranged from a mild stimulation by amitrole at 50  $\mu\text{g/ml}$  to almost complete inhibition of growth by other herbicides at varying concentrations. *R. meliloti* and *R. leguminosarum* were less sensitive to herbicides than *R. trifolii*, *R. lupini* and *R. japonicum*. Of the herbicides tested, dalapon was found to have a marked inhibitory effect on the growth of *Rhizobium* spp. in culture, but the herbicide had no noticeable effect on the nodulation of lucerne under bacteriologically controlled conditions. Amitrole, on the other hand, delayed nodulation, decreased the number of nodules and plant length even at 1  $\mu\text{g/ml}$  level, while in



culture medium, the herbicide, in fact, stimulated the growth of rhizobia, suggesting that growth stimulation/inhibition of rhizobia had no direct connection with the extent of root nodulation.

Nodulation of soybean was improved when seed was inoculated with *R. japonicum* as well as *Azospirillum brasilense*. Similar synergistic effects were demonstrated when leguminous plants were inoculated with their respective rhizobia in the presence of *Glomus fasciculatum*, a vesicular-arbuscular mycorrhizal (VAM) fungus which commonly occurs on the roots of legumes. The VAM fungus improves phosphorus nutrition in legumes, which is so essential for nitrogen fixation. The benefits to legumes of this dual inoculation with *Rhizobium* + VAM fungus is being exploited to improve yields.

The quantity of nitrogen fixed by leguminous crops under field conditions has always been estimated grossly. Accurate estimation of  $N_2$  fixation by chickpea plants (*Cicer arietinum*) was done in four successive field trials using labelled ammonium sulphate; the results obtained indicated that this legume can fix 63 kg N/ha under field conditions.

Other important contributions include survey and serological studies of *Rhizobium* strains in Indian soils and studies on the interaction of *Rhizobium* with root calli. An important area of investigation was the isolation and testing of *Arachis* rhizobia from wild *Arachis duranensis*.

Providing workable and efficient criteria for the production and supply of quality rhizobial inoculants to the cultivators is an integral part of a practising rhizobiologist.

For the first time in a third world country, quality control measures were devised by IARI which enabled the Indian Standards Institution to formulate effective quality control standards in the country.

### **Associative Symbiosis in Grasses and Millets**

For the first time in India, several strains of *Azospirillum*, a microaerophilic associative  $N_2$  fixing bacterium, were isolated from roots of rice, maize, common grasses, barley, sorghum, pearl millet and wheat and tested for field performance under the All India Coordinated Crop Improvement projects of the ICAR. The results of these field trials conducted for four years have shown that seed inoculation with this new biofertilizer can increase crop yields of bajra, sorghum and ragi. These crops generally receive very little agronomic inputs, especially fertilizer nitrogen.

### **Interaction of Nitrogen Fixing Bacteria with VAM Fungi**

Vesicular-arbuscular mycorrhizal (VAM) fungi are endosymbionts in legumes as well as non-legumes and function as living appendages in transporting nutrients, especially phosphorus from soil to the root system.

For the first time, it has been shown that dual inoculation with *Azospirillum brasilense* and VAM fungi increases the yield of millets by providing nitrogen as well as phosphorus nutrition to the plants. Similarly, with the help of labelled ammonium sulphate, it has been shown that greater accumulation of  $^{15}N$  label takes place in VAM inoculated chickpea plants when inoculated with VAM alone or in combination with *Rhizobium* cultures. This approach has given a lead in

providing a suitable blend of nitrogen and phosphorus mobilizing microorganisms as inoculants in crop cultivation.

### Future Direction

Undoubtedly, there exist many more bacteria within plants which are capable of providing nitrogen for plant growth, as evidenced by the huge growth of grasses and other plants, even on sand-dunes having no nutritive value. Some of these endophytic bacteria may be obligate symbionts and one has to devise ways and means to culture them on plates. If one can transfer 'nif' genes from bacteria to VAM fungi, both the phosphorus and nitrogen needs of plants can be met. Research efforts in these directions by future generation of scientists are likely to provide exciting results.

### Selected Publications

1. Subba Rao N S, Fluorescence phenomenon in Fusarirose wilt of cotton, *J Indian bot Soc*, **33** (1954) 443-45.
2. Andal R, Bhuvaneswari K & Subba Rao N S, Root exudates of paddy, *Nature, Lond*, **173** (1956) 1063.
3. Subba Rao N S, Bidwell R G S & Bailey D L, The effect of rhizoplane fungi on the uptake and metabolism of nutrients by tomato plants, *Can J Bot*, **39** (1961) 1759-64.
4. Subba Rao N S, Bidwell R G S & Bailey D L, Studies of rhizosphere activity by the use of isotopically labelled carbon, *Can J Bot*, **40** (1962) 203-12.
5. Lakshmi Kumari M, Singh C S & Subba Rao N S, Effect of salinity and alkalinity on the early phases of infection in lucerne (*Medicago sativa*), *Plant & Soil*, **40** (1974) 261-66.
6. Subba Rao N S, Lakshmi Kumari M & Singh C S, Salinity and alkalinity in relation to legume-*Rhizobium* symbiosis, *Proc Indian natn Sci Acad*, **40B** (1974) 544-47.
7. Lakshmi Kumari M, Biswas A, Vijayalakshmi K, Narayana H S & Subba Rao N S, Effect of certain water soluble herbicides on legume-*Rhizobium* symbiosis, *Proc Indian natn Sci Acad*, **40B** (1974) 528-34.
8. Lakshmi Kumari M, Kavimandan S K & Subba Rao N S, Occurrence of nitrogen fixing *Spirillum* in roots of rice, sorghum, maize and other plants, *Indian J exp Biol*, **4** (1976) 638-39.
9. Subba Rao N S, Tilak KVBR, Lakshmi Kumari M & Singh C S, Response of a few economic species of graminaceous plants to inoculation with *Azospirillum brasilense*, *Curr Sci*, **48**(3) (1979) 133-34.
10. Singh C S & Subba Rao N S, Associative effect of *Azospirillum brasilense* with *Rhizobium japonicum* on nodulation and yield of soybean (*Glycine max*), *Plant & Soil*, **53** (1979) 387-92.
11. Dewan G I & Subba Rao N S, Seed inoculation with *Azospirillum brasilense* and *Azotobacter chroococcum* and the root biomass of rice (*Oryza sativa* L), *Plant & Soil*, **53** (1979) 295-302.
12. Subba Rao N S, *Soil microorganisms and plant growth* (Oxford and IBH, New Delhi) 1977.
13. *Recent advances in biological nitrogen fixation*, edited by N S Subba Rao (Oxford and IBH, New Delhi and Edward Arnold, UK) 1979.
14. Subba Rao N S, *Biofertilizers in agriculture* (Oxford and IBH, New Delhi) 1982.
15. *Advances in agricultural microbiology*, edited by N S Subba Rao (Oxford and IBH, New Delhi and Butterworths, UK) 1982.
16. *Current developments in biological nitrogen fixation*, edited by N S Subba Rao (Oxford and IBH, New Delhi and Edward Arnold, UK) 1986.



## M S Swaminathan

The major research contributions of Swaminathan are discussed below in brief.

### **(1) Origin, Cytogenetics and Species Interrelationships in the Genera *Solanum*, *Triticum* and *Oryza***

During the period 1947-1960, detailed investigations were carried out on species differentiation, cytogenetic affinity and the role of polyploidy in evolution and breeding. From a systematic study of genetic relationships in tuber-bearing sub-species of *Solanum*, it was concluded that the cultivated potato varieties had their origin from sub-species *andigenum* of *S. tuberosum*. The tetraploid potato *S. tuberosum* is a segmental allopolyploid. Cryptic structural differences in chromosomes are largely responsible for partial sterility in inter-specific crosses.

An effective technique was developed for inducing polyploidy in tuber-bearing species of *Solanum*. Using this technique, genes for frost resistance were transferred from the wild species *S. acaule* into *S. tuberosum*. A simple technique was developed for overcoming barriers to hybridization caused by the inhibition of pollen tube growth in the style of the female parent in crosses among some diploid *Solanum* species<sup>1-5</sup>.

In wheat, considerable work aimed at understanding the role of macro- and micro-mutations in sub-specific

differentiation of *Triticum aestivum* was done. Also, the structure of Q locus in chromosome 5A was studied in detail. Based on an understanding of the genetics of different characters, methods of screening for spontaneous and induced mutations were standardized. In hexaploid wheat, Dr E R Sears of the University of Missouri, USA, had developed a complete set of monosomics in the variety Chinese Spring. This opened up opportunities for a detailed study of linkage groups in this hexaploid plant. Unfortunately, however, Chinese Spring had a very poor combining ability and, therefore, the monosomic series in this variety did not prove useful for the transfer of alien genes into commercially successful varieties of bread wheat. Hence, a complete set of monosomics was developed in the Punjab wheat variety, C.591<sup>6,7</sup>.

Cytogenetic and breeding studies in rice included the search for diverse genes for semi-dwarfing, shuttle breeding under two contrasting environments, search for male sterile lines for the purpose of developing hybrid rice and the collection, conservation and classification of rice genetic resources from the Northeastern Himalayas.

In all the crops investigated, steps were taken to collect, conserve and utilize wild germplasm and primitive cultivars. The extensive work done on genetic

conservation over a period of 30 years formed the basis for the Presidential Address at the XV International Congress of Genetics held in New Delhi in December 1983<sup>8</sup>.

## **(2) Induced Mutagenesis**

Since spontaneously occurring mutations provide an important source of new variability, experiments were started in wheat in 1955 on the induction of mutations of plant breeding as well as phylogenetic value. In order to gain some degree of control over the process of induced mutagenesis, considerable work was done with both ionizing radiations and chemical mutagens. Treatments were given during different phases of DNA synthesis in barley. Also, the effects of various modifying factors and pre- and post-treatments were studied. The relationship between polyploidy and radio-sensitivity was studied in detail. During this research, it was found that certain characters like awning and grain colour could be changed in bread wheat, thereby making the induced mutation technique serve a purpose similar to backcrossing for achieving changes in one character while keeping all the other parental traits intact. Another important finding related to the mutagenic properties of some widely used vegetable oils<sup>6,9</sup>.

Cytogenetic studies of the effects of radiations on cells and whole plants revealed that radio-mimetic compounds are produced in irradiated seeds and substrates, which, in turn, could enhance the natural mutation frequency in barley and *Drosophila*. Chromosome aberrations were also caused by the secondary effects of radiations. A series of papers using a wide range of test organisms published between 1958 and 1965 aroused

considerable interest in view of their implications for the assessment of the wholesomeness of irradiated food material. These findings led to the inclusion of genetic criteria in the assessment of wholesomeness of irradiated food products<sup>10,11</sup>.

## **(3) Raising the Ceiling of Yield in Wheat and Rice**

Based on an analysis of the constraints responsible for the gap between potential and actual experimental yields in bread wheat grown under irrigated conditions, it was proposed in 1961 that the introduction of the Norin dwarfing genes is essential for the development of high yielding and management responsive varieties of wheat. This was because the Norin dwarfing genes had no adverse effect on panicle size and spikelet and grain number. This led to the introduction of a wide range of spring wheat materials containing the Norin dwarfing genes from the wheat breeding programme of Dr N E Borlaug in Mexico as well as dwarf winter wheat material from Dr O Vogel of USA. Soon after this material was received in IARI in 1963, a five-pronged strategy was introduced for the rapid identification, development and popularization of wheat varieties capable of responding well to irrigation and mineral fertilizer application. These are:

(a) Direct introduction of varieties from Mexico which performed well under the growing conditions in India.

(b) Selection of strains from advanced breeding material received from Mexico.

(c) Hybridization between well adapted tall strains and semi-dwarfs having the Norin dwarfing genes.



(d) Transfer of the dwarfing gene(s) to *Triticum durum* cultivated in Central and Peninsular India.

(e) Mutational rectification of undesirable traits.

Suitable methods of technology transfer, including the organization by scientists of national demonstrations in small farmers' fields, were developed in 1965. These demonstrations had a dramatic impact on the minds of farmers and resulted in the rapid spread of high yielding strains. Thus, from 4 hectares in 1964, the semi-dwarf of varieties of wheat occupied over 4 million hectares in 1970.

In rice, the emphasis was on the transfer of Dee-gee-wu-gen gene into varieties with good grain quality and desirable pest resistance. The shuttle breeding approach under two diverse environments led to the development of varieties like Pusa-2-21 characterized by very wide adaptation<sup>12,13</sup>.

#### **(4) Management of Adverse Weather Conditions**

Since considerable fluctuations take place in crop production as a result of variability in monsoon behaviour, work was done on different aspects of drought management. Three major approaches were introduced for minimizing the adverse impact of both deficits and skewed distribution of rainfall: (a) alternative cropping patterns and contingency plans to suit different weather probabilities, (b) standardization of crop life-saving and regeneration techniques, and (c) compensatory production programmes in favourable areas with adequate soil moisture.

The concept of contingency planning led to the initiation of programmes for building grain reserves of alternative crops<sup>14</sup>.

#### **(5) Biotechnology and Genetic Engineering**

In many crop plants like rice and wheat, continuous research becomes essential even to defend the production gains already made. This is because new problems of soil stresses (toxicities and deficiencies) and of pest epidemics arise under conditions of intensive agriculture. Therefore, continuous identification and transfer of genes for resistance or tolerance to pests and pathogens, including new biotypes and to soil stresses, such as alkalinity and salinity, is essential for sustaining agricultural progress. For this purpose, the scope offered by somaclonal and gametoclonal variation can be tapped. Extensive research in this field has been initiated in rice. Also, there is need to develop suitable vectors for the transfer of genetic material in monocots. Today, hybridization can be attempted at the sexual, parasexual and molecular levels. Similarly, the conservation of genetic resources can be done at the level of populations, individual genotypes, cells and DNA. This is the main focus of current work<sup>8,15</sup>.

#### **Selected Publications**

1. Swaminathan M S, Einige Verfahren fur die Verwendung wilder *Solanum* arten zu suchtzwecken, *Zuchter*, **20** (1950) 358-60.
2. Swaminathan M S, Nature of polyploidy in some 48-chromosome species of the section *Tuberarium* genus *Solanum*, *Genetics*, **39** (1954) 56-76.
3. Swaminathan M S, Overcoming cross-incompatibility among some Mexican diploid species of *Solanum*, *Nature, Lond*, **176** (1955) 887-88.
4. Swaminathan M S, The origin of the early European potato—Evidence from Indian potato varieties, *Indian J Genet*, **18** (1958) 8-15.

5. Swaminathan M S & Magoon M L, The origin and cytogenetics of the commercial potato, *Adv Genet*, **10** (1961) 217-56.
6. Swaminathan M S Mutational analysis of the hexaploid Triticum complex, *Proc 2nd Int Wheat Genetics Symp*, Lund, 1963, *Hereditas*, **2** (1966) 418-38.
7. Swaminathan M S, Chopra V L, Joshi B C & Singh D, Development of monosomic series in an Indian wheat and isolation of nullisomic lines, *Wheat Inf Serv*, **27** (1968) 19-20.
8. Swaminathan M S, Genetic conservation: Microbes to man, *XV International Congress of Genetics*, New Delhi, 12-21 December 1983, 4-30.
9. Swaminathan M S & Natarajan A T, Cytological and genetical changes induced by vegetable oils in *Triticum* species, *J Heredity*, **50** (1959) 177-87.
10. Swaminathan M S, Chopra V L & Bhaskaran S, Cytological aberrations in barley embryos cultured in irradiated potato mash, *Radiat Res*, **16** (1962) 182-88.
11. Swaminathan M S, Nirula S, Natarajan A T & Sharma R P, Mutation incidence in *Drosophila melanogaster* reared on irradiated medium, *Science*, **41** (1963) 637-38.
12. Swaminathan M S, Past, present and future trends in tropical agriculture in *Perspectives in world agriculture* (Commonwealth Agricultural Bureaux) 1980, 1-47.
13. Swaminathan M S, Plant breeding in preparation for the 21st century, *Proc Indian natn Sci Acad*, **B48** (1982) 1-18.
14. Swaminathan M S, Climate and agriculture, in *Climate and development*, edited by Asit K Biswas (Tycooly International Publishing Ltd, Dublin) 1984, 65-95.
15. Swaminathan M S, DNA in medicine—Agricultural production, *Lancet* (8 December 1984) 1329-32.



## J N S Yadava

Gastroenteritis of man and animals associated with *Escherichia coli* and *Salmonellae* has been investigated by workers in great detail, because the two groups of bacteria occur commonly in the intestine of man, animals and birds without any apparent symptoms. For instance, human enteropathogenic forms of *Salmonella* may exist in more than 32 species of domestic animals. Comparatively less attention has been given in India to the magnitude of drug resistance in these bacteria.

### New Approaches

Epidemiological surveys and bacteriological analytical tests were conducted by us with reference to gastroenteritis outbreaks<sup>1</sup> in man and animals in India. These surveys were aimed primarily at developing improved and reliable means of detecting the origin and spread of drug resistance in incriminating enteropathogenic bacteria involved in zoonotic diseases that man and his animals readily acquire from each other. Specific and rapid methods of classification and identification (typing) of enteropathogenic *E. coli* based on sugar fermentation, biochemical reactions, antigenic characters, nutritional requirements, production of colicins, haemolysins, enterotoxins, haemagglutinins, lysogenicity, phage susceptibility

and bacteriolytic activity of normal serum have been developed<sup>2-9</sup>.

### Drug Resistance

Sensitivity testing of isolated *E. coli* strains against 17 commonly prescribed antibiotics revealed that 85% of the strains were resistant to one or more antibiotics. The overall incidence of resistance individually or in combinations with other antibiotics was recorded as the highest (in the range 34-70%) against cloxacillin, streptomycin, tetracycline, penicillin, carbencillin and oxytetracycline. Incidence of amoxycillin, doxycycline, chloramphenicol, Ampicillin and rifampicin resistant strains was somewhat low (26-33%). Resistance against cephalosporidine, cephalaxin and septran was found to be low (11-17%), while it was the lowest against nalidixic acid, kanamycin and nitrofurantoin (0.9-7%). The incidence of multiple resistance was more as compared to single or double resistance. Such resistance is generally reported as plasmid encoded (extra-chromosomal genetic elements) and its relevance was investigated<sup>5,9,10</sup>.

### Infectious Drug Resistance

Forty-nine per cent of the *E. coli* strains investigated were shown to transfer by conjugation their resistance status singly or en bloc. The frequency of transfer was

---

Assistant Director, Central Drug Research Institute, Lucknow-226001; Residence : 52, New Hyderabad, Dr Deen Dayal Gupta Marg, Lucknow-226001.

recorded more in penicillin, followed by ampicillin, streptomycin and tetracycline. About 24% of the strains were also found to transfer their resistance in part or en bloc to *Salmonella typhimurium*. The incidence of antibiotic resistance transfer was found to be low through transduction mediated by phage  $P_1$  as compared to conjugation<sup>9-12</sup>.

Studies in inter- and intra-generic transfer of drug resistance in experimental animals showed that the frequency of R-factor transfer was greater in the gut of suckling rat, from *E. coli* to *S. typhimurium*, than from *E. coli* strains to non-plasmid bearing recipient *E. coli* K-12. The overall incidence of antibiotic resistance transfer by *E. coli* to both recipients<sup>12</sup> was very high (23.6%).

### Plasmids in Drug Resistance

Several physico-chemical agents (proteins, DNA and RNA synthesis inhibitors) were used for the elimination of plasmids associated with drug resistance. Transfer of penicillin, ampicillin, tetracycline and chloramphenicol was completely checked at acidic (5.5) as well as alkaline pH (8.5). The incidence of *in vitro* resistance transfer was higher as compared to *in vivo* transfer, which could be due to the presence of bile in the intestinal tract. Bile salts (sodium taurocholate and sodium thioglycolate) were also significantly effective in blocking the transfer of resistance in multiple resistant strains compared to double or single resistant strains. Sodium taurocholate in general was more effective in inhibiting transfer of resistance than sodium thioglycolate<sup>10,12-14</sup>.

Among the inhibitors used, acridine orange was more effective than ethidium

bromide and acriflavine. Rifampicin and mitomycin 'C' were found highly effective and eliminated most of the resistant plasmids from resistant *E. coli* strains to a variable degree. Nalidixic acid also eliminated all types of R-plasmids, but it was particularly active against double antibiotic resistant strains. Among the protein synthesis inhibitors used, gentamicin and kanamycin were found most effective in eliminating the R-factor (plasmid) from the resistant bacteria. Chloramphenicol was not found to be as effective as gentamycin; similarly, erythromycin also proved to be a poor eliminating agent. In general, DNA inhibitors were found most effective among all curing agents used in these studies<sup>13,14</sup>.

### Spontaneous Disappearance of Plasmids

A high frequency of spontaneous loss of plasmids was recorded after long storage and continuous subculture of bacterial strains in drug-free media. The rate of spontaneous loss of drug resistance was very high among kanamycin and nitrofurantoin resistant strains (98 and 66% respectively). The loss of penicillin, chloramphenicol and ampicillin resistance was also recorded, but at a lower frequency (43.7, 40.6 and 37% respectively). Complete loss of resistance was also recorded in 21% of the strains. Tetracycline and streptomycin resistance markers were found to be most stable<sup>15</sup>.

### Virulence in Bacteria: Evidence of Plasmid Control

That the plasmids ( $Col^+$ ,  $R^+$  and  $Ent^+$ ) may control aspects of bacterial pathogenicity and virulence has been suggested by the results of the following



investigations. Colicinogeny (Col I and B + M) was transferred by conjugation from *E. coli* (Col<sup>+</sup> donor) to non-colicinogenic *E. coli* K-12 (recipient), normally unable to survive in animal intestine. Such converted (genetically engineered) strains were shown to compete, survive and multiply very successfully in the adverse environmental conditions of the intestine. If the engineered bacteria are deprived of the plasmid by eliminating it with acridine<sup>13</sup>, the ability to survive in intestinal environment is lost<sup>16</sup>.

Plasmids controlling multiple drug resistance and serum resistance associated with virulence and pathogenicity in bacteria originating in animal intestines can be transferred readily to human *E. coli* and *Salmonella* strains by processes of conjugation and transduction (mediated through phase P<sub>1</sub>). Such genetically engineered bacteria can be cloned and have shown the capacity to survive for a longer period in the animal intestines. Elimination of these plasmids by chemical treatment (acridine) led to return to normal survival time<sup>10-12</sup>.

Similar elimination of 'Ent' plasmid from the engineered bacteria (Ent<sup>+</sup>) led to its inability to produce enterotoxins, as could be judged by the ileal loop method unlike the uneliminated Ent-plasmid parent<sup>11,17,18</sup>.

The enteropathogenic *E. coli* are frequently found enterotoxigenic as recorded by ileal loop methods. The ETEC strains were frequently found resistant to one or more antibiotics. High incidence of antibiotic resistance among ETEC strains was recorded as compared to non-ETEC strains. These strains were mostly multiple resistant, showing different resistance pattern. ST<sup>+</sup> strains were found more

resistant to antibiotics than strains producing LT or LT-ST. This shows a strong correlation between R-plasmids and ST producing plasmids. ETEC strains isolated from UTI and GTI cases were generally multiple resistant as compared to non-ETEC strains<sup>19</sup>.

The transferability of enterotoxigenicity along with drug resistance to non-pathogenic *E. coli* strains is of much interest in understanding the role of such plasmids in bacterial pathogenicity and virulence. About 35% of ETEC strains co-transferred their antibiotic resistance and Ent<sup>+</sup> plasmid to *E. coli* K-12 recipient *in vitro*. Transfer of resistance from single to multiple antibiotics in different combinations with enterotoxigenicity was also recorded. None of the antibiotic sensitive toxigenic donors could transfer its toxigenicity. This may be due to the absence of a sensitive selection marker; 'Ent'-transmission can only be demonstrated when it occurs at a very high rate. The study suggested that genes for enterotoxin carried together, conferring virulence and pathogenicity to the host bacteria<sup>20</sup>.

ETEC must possess accessory virulence properties in addition to produce LT or ST to cause diarrhoeal disease. The best characterized virulence properties are colonization factors which allow ETEC to adhere to specific receptors on enterocytes of the proximal small intestinal mucosa, thereby giving the potent defence mechanism against peristaltic movements. All specific colonization factors identified so far in animal or human ETEC strains have turned out to be fimbriae (also referred to as pili). Bacteria bearing these fimbriae cause mannose-resistant haemagglutination of erythrocytes of

certain animal species, providing a simple screening test to detect their presence. The naturally occurring LT<sup>-</sup>/ST<sup>+</sup> and LT<sup>+</sup>/ST<sup>-</sup> strains isolated from cases of diarrhoea are indeed pathogenic. They cause diarrhoea and colonize the intestine<sup>21</sup>.

There exists a close correlation between the presence of specific fimbriae and certain 'O' sero groups. CFA/I was mostly associated with LT<sup>+</sup> ETEC strains (46%) as compared to ST<sup>+</sup> (14%) and LT<sup>+</sup>-ST<sup>+</sup> (11%). CFA/I predominated in enteropathogenic sero groups 04, 05, 011, 012, 025 and 060.

Enteropathogenic *E. coli* (EPEC) strains showed a wide variety of haemagglutination patterns, both mannose sensitive (MSHA) and mannose resistant (MRHA). Few strains showed variable haemagglutinins as they agglutinated with erythrocytes of different species, which may be due to multiplicity of bacterial adhesins. MRHA with human type A erythrocytes was observed mostly in ETEC strains which strongly suggests their virulent property in diarrhoeal diseases. The study revealed that 56% of the strains could not haemagglutinate with erythrocytes of any animal species either MS or MR, although they were all isolated from acute urinary tract infections. In such strains, some other plasmids coding for colicins, haemolysins, etc., might be influencing the pathogenicity<sup>18</sup>.

Therefore, the virulence and pathogenicity of bacteria do not always reflect the specific serotypes only, but are directly related to the various virulence plasmids present in the bacterium. Such plasmids encoded virulence factors are independent of serotypes, because determinants of surface antigens are chromosomally mediated. These virulence

factors have direct or indirect influence on R-plasmids also. It may be concluded that a drug resistant strain is more pathogenic than a drug sensitive one, irrespective of its serotype, because in this study, R-plasmids have shown a strong correlation with plasmids which encode for virulence and pathogenicity<sup>20</sup>.

In enteropathogenic strains, it has been shown that haemolysins, colicins and a few surface antigens (K88, K89, K99) contribute to virulence of bacteria. It has been proved that plasmid borne genes linked to antibiotic resistance and colicin can code for serum resistance of *E. coli*. A very close association of colicins haemolysins, enteropathogenic serotypes with serum and antibiotic resistance was found.

### Future Research

Plasmids associated with drug resistance and the factors associated with the production of enterotoxins and colicins in human pathogenic *E. coli* and *Salmonella* have been detected at a rather high frequency in animal intestines. It would, therefore, seem that identification and knowledge of specific extra-chromosomal genetic elements in the isolated bacteria, coupled with serotyping and phage typing, will contribute materially to refinement of the existing technique of tracking the source and origin of zoonotic diarrhoeal infections, thus helping in developing better management and control of zoonotic gastroenteritis. Secondly, intelligent search for specific chemicals with higher margin of safety can now be expected to form a rational programme of research in the coming years for the development of safe drugs and vaccines against bacterial gastroenteritis.



### Selected Publications

1. Yadava J N S, Rajami H B, Ansari M Q & Saxena V K, A fatal outbreak of gastro-enteritis in adult and young animals due to *Escherichia coli* infection and its management, *Indian vet Med J*, **8**(2) (1984) 51.
2. Ahmad S & Yadava J N S, Haemolysin production among *Escherichia coli* strains isolated from clinical cases of man and animals, *Indian vet Med J*, **4** (1980) 149.
3. Ahmad S & Yadava J N S, Transmissible antibiotic resistance among haemolytic strains of *Escherichia coli* and correlation with their pathogenicity, *Indian vet Med J*, **7** (1983) 70.
4. Ansari M Q & Yadava J N S, Colicinogeny and lysogeny among clinical isolates of *E. coli* from human and animal sources, *Indian J anim Sci*, **51** (1980) 664.
5. Ansari M Q, Yadava J N S, Goel R & Rajani H B, Increasing incidence of antibiotic resistance among *Escherichia coli* strains and its transferability to *Salmonella typhimurium*, *Indian vet J*, **61** (1984) 1001.
6. Yadava J N S & Ansari M Q, Evaluation of Routine Test Dilution in phage typing of *Escherichia coli*, *Indian vet Med J*, **3** (1979) 224.
7. Yadava J N S & Gupta B M, Antigenic analysis and biochemical characters of strains of *Escherichia coli* commonly associated with human diarrhoea, isolated from sporadic cases of gastroenteritis in local population of domestic animals, *J gen appl Microbiol*, **15** (1969) 197.
8. Yadava J N S & Gupta B M, Studies on colicinogeny, lysogeny and phage susceptibility of 153 strains of *Escherichia coli* isolated from gastro-enteritis of domestic animals, *Indian J anim Sci*, **41** (1971) 428.
9. Yadava J N S, Ahmad S & Rajani H B, Production of  $\beta$ -lactamases by clinical isolates of *Escherichia coli* and their possible role in resistance to penicillin and ampicillin, *Indian J anim Sci*, **54** (1984) 944.
10. Yadava J N S, Ansari M Q and Goel R, Transfer of antibiotic resistance by transduction and conjugation in clinical isolates of *E. coli*, *Indian J anim Sci*, **53** (1983) 892.
11. Yadava J N S, Goel R & Ansari M Q, Colorimetric serum resistance assay of drug resistant *E. coli* strains, *Curr Sci*, **51** (1982) 1041.
12. Yadava J N S, Goel R & Ansari M Q, Auto-transferable R-plasmids in *E. coli* strains and their transfer to *S. typhimurium*, *Indian vet Med J*, **6** (1982) 242.
13. Goel R & Yadava J N S, Curing effect of rifampicin and mitomycin-C on R-plasmids of *E. coli* strains, *Indian vet Med J*, **5** (1981) 165.
14. Goel R, Ansari M Q & Yadava J N S, Effects of sodium taurocholate and sodium thioglycolate on the conjugal transfer of R-plasmids in *Escherichia coli* strains, *Indian vet Med J*, **8** (1984) 14.
15. Yadava J N S, Ansari M Q, Goel R & Rajani H B, Spontaneous loss of drug resistance under laboratory conditions in clinical isolates of *Escherichia coli*, *Indian J med Res*, **80** (1984) 148.
16. Goel R, Yadava J N S & Ansari M Q, Increased survival of *Escherichia coli* K-12 harbouring col-plasmid in intestinal tract of mice, *Indian vet Med J*, **4** (1980) 174.
17. Saxena V K & Yadava J N S, Evaluation of animal models for detection of heat stable enterotoxin of *E. coli*, *Indian vet Med J*, **6** (1982) 118.
18. Saxena V K & Yadava J N S, Haemagglutinins and enterotoxins among *Escherichia coli* strains from human urinary tract infections, *Indian J med Res*, **81** (1985) 35.
19. Saxena V K & Yadava J N S, Enterotoxigenicity and antibiotic resistance among human clinical isolates of *Escherichia coli*, *Indian J anim Sci*, **55** (1985) 625.
20. Saxena V K & Yadava J N S, Incidence of enterotoxin production and colonization factor among various somatic antigen group of *Escherichia coli* strains, *Indian J med Res*, **82** (1986), In press.
21. Yadava J N S, Saxena V K & Ansari M Q, Plasmid coding for haemolysins, enterotoxins and haemagglutinins and their role in virulence and pathogenicity of *Escherichia coli*, *Indian J Path Microbiol*, **29** (1986), in press.

## Index : Volume 2

- Abrol I P 1421  
 Abrol Y P 845  
 Adiga P R 1305  
 Agarwal S C 1125  
 Agarwala S C 847  
 Akhtar Husain 1425  
 Anand B K 1129  
 Ananthakrishnan T N 1023  
 Appaji Rao N 1310  
 Asana R D 851  
 Awasthi D D 855  
 Bachhawat B K 1315  
 Balakrishnan Nair N 1025  
 Banerjee Sachchidananda 1134  
 Barnabas John 1318  
 Bhaduri Amar Nath 1323  
 Bhargava K S 859  
 Bhargava P M 1325  
 Bhat J V 1140  
 Bhatia I S 1428  
 Bhatia M L 1028  
 Bilgrami K S 862  
 Bir S S 864  
 Biswas B B 1329  
 Bose M N 870  
 Burma D P 1332  
 Chatterjee S N 1146  
 Chopra S R K 1150  
 Chopra V L 874  
 Choudhury Bishwajit 1432  
 Chowdhury A B 1153  
 Das Gupta C R 1156  
 Das Gupta N N 1337  
 Das Gupta S N 878  
 Dass C M S 1031  
 Dastur Dorb K 1161  
 Datta Asoke G 1341  
 Datta Munshi J S 1034  
 De Rajat 1435  
 Deo M G 1146  
 Deodikar G B 1438  
 Desikachary T V 882  
 Dhawan B N 1167  
 Dogra P D 1442  
 Dominic C J 1039  
 Dutta G P 1042  
 Esh G C 1344  
 Gadgil Madhav 1447  
 Ganapathi K 1348  
 Gangulee H C 885  
 Ganguly H C 1171  
 Ganguly J 1352  
 Ghildyal B P 1450  
 Ghosh Asok 1046  
 Ghosh T K 1175  
 Gill K S 1453  
 Gopalan C 1178  
 Gopinath N 1181  
 Gupta B M 1457  
 Gupta P K 887  
 Guraya S S 1049  
 Heilig Robert 1184  
 Ishwar Prakash 1056  
 Jagannathan V 1355  
 Jain H K 1463  
 Jain S K 890  
 Jhingran V G 1058  
 Johri B M 893  
 Kanungo M S 1062  
 Kanwar J S 1466  
 Kapil R N 899  
 Kedarnath S 1470  
 Khan Abrar M 1475  
 Khoshoo T N 903  
 Khush Gurdev S 1479  
 Krishna Murti C R 1357  
 Krishna Swarup 1065  
 Kundu B C 908  
 Lakhanpal R N 911  
 Lal K B 1483  
 Laumas K R 1186  
 Luthra Usha K 1191  
 Madhava Reddy G 1484  
 Maitra P K 1360  
 Malik C P 915  
 Manna G K 1068  
 Mathur K S 1195  
 Mehra P N 919  
 Mehrotra K N 1073  
 Misra R 926  
 Modi V V 1489  
 Mohan Ram H Y 929  
 Mookerjee Sviatosh 1078  
 Moudgal N R 1362  
 Mukherjee A S 1081  
 Mukherjee S K 1492  
 Murty B R 1494  
 Murty Y S 935  
 Nath M C 1365  
 Notani N K 1371  
 Padmanaban G 1374  
 Pal B P 1499  
 Pal R K 1198  
 Pandeya S C 940  
 Pant D D 945  
 Paroda R S 1501  
 Parthasarathy N 1507  
 Pillai M K K 1085  
 Prasad M R N 1088  
 Prem Narain 1510  
 Prihar S S 1514  
 Puri V 947  
 Raizada M B 953  
 Ramachandran G N 1377  
 Ramachandran L K 1383  
 Rama Das V S 954  
 Ramahrishanan P S 958  
 Ramakrishnan T 1203  
 Ramalingaswami V 1207  
 Ramamurthi B 1210  
 Ramanathan N 1388  
 Ramaswami L S 1092  
 Ramiah 1518  
 Ranadive Kamal J 1212  
 Randhawa M S 1522  
 Randhawa N S 1524  
 Rao S R V 1096  
 Rao N G P 1528  
 Rao V S R 1392  
 Ray A P 1216  
 Raychaudhury Satya P 1  
 Raychaudhury Syama P  
 Reddy C R R M 1221  
 Roonwal M L 1098  
 Roy B B 1226  
 Roy R P 961  
 Sadasivan T S 965  
 Sadhu D P 1540  
 Saha A K 1232  
 Saksena S B 969  
 Salim Ali 1101  
 Sarkar J K 1235  
 Sarma Y S R K 972  
 Sasisekharan V 1397  
 Sastry P S 1401  
 Sathyanesan A G 110  
 Seal S C 1239  
 Sen A B 1245  
 Sen H G 1247  
 Sen P K 1251



- Sen S P 976  
Shah J J 979  
Shah V C 1107  
Sharma A K 982  
Sharma Archana 986  
Sharma G P 1109  
Sharma K N 1255  
Sharma V P 1111  
Shastry S V S 1542  
Singh B N 990  
Singh J S 994  
Sinha A K P 1257  
Sinha S K 997
- SivaRaman C 1404  
Sohi H S 1000  
Sreenivasan A 1407  
Srinivasan S 1409  
Sriramachari S 1261  
Srivastava Har Dayal 1114  
Srivastava P N 1118  
Srivastava U S 1120  
Subbarao N S 1545  
Sukhatme P V 1267  
Sundaram K 1272  
Surange K R 1004  
Swaminathan M 1276
- Swaminathan M S 1548  
Talwar G P 1279  
Tandon P N 1286  
Tandon R N 1007  
Thirumalachar M J 1009  
Thomas Joseph 1412  
Trivedi B S 1014  
Vaidyanathan C S 1416  
Valiathan M S 1291  
Venkataraman G S 1017  
Wadia N H 1293  
Wahi P N 1296  
Yadav J N S 1552

## Cumulative Index : Volumes 1 and 2

(Volume number is given in bold followed by page number in italics)

- |   |                                     |                                   |
|---|-------------------------------------|-----------------------------------|
| Abraham K P <b>1</b> : 575              | Biswas S N <b>1</b> : 129           | Dhar N R <b>1</b> : 389           |
| Abrol I P <b>2</b> : 1421               | Biswas Sukumar <b>1</b> : 131       | Dhawan B N <b>2</b> : 1167        |
| Abrol Y P <b>2</b> : 845                | Bomford G <b>1</b> : 730            | Dinesh Mohan <b>1</b> : 610       |
| Adiga P R <b>2</b> : 1305               | Bose A <b>1</b> : 134               | Divatia A S <b>1</b> : 158        |
| Agarwal G S <b>1</b> : 119              | Bose A K <b>1</b> : 350             | Dogra P D <b>2</b> : 1442         |
| Agarwal S C <b>2</b> : 1125             | Bose M N <b>2</b> : 870             | Dominic C J <b>2</b> : 1039       |
| Agarwala S C <b>2</b> : 847             | Bose Mihir K <b>1</b> : 731         | Doss K S G <b>1</b> : 391         |
| Agarwala U C <b>1</b> : 323             | Burma D P <b>2</b> : 1332           | Dumir V C <b>1</b> : 12           |
| Ahluwalia J C <b>1</b> : 327            | Buti Bimla <b>1</b> : 140           | Dutt S <b>1</b> : 395             |
| Ahmad F <b>1</b> : 715                  | Chadha Mohindra S <b>1</b> : 355    | Dutta A K <b>1</b> : 161          |
| Akhtar Husain <b>2</b> : 1425           | Chakrabarty S K <b>1</b> : 6        | Dutta G P <b>2</b> : 1042         |
| Anand B K <b>2</b> : 1129               | Chakravarti R N <b>1</b> : 359      | Dutta Majumdar D K <b>1</b> : 614 |
| Ananthakrishnan R <b>1</b> : 717        | Chakravarty R B <b>1</b> : 595      | Dutta Roy S C <b>1</b> : 618      |
| Ananthakrishnan T N <b>2</b> : 1023     | Chakravorty Animesh <b>1</b> : 364  | Esh G C <b>2</b> : 1344           |
| Anantharaman T R <b>1</b> : 578         | Chakravorty D <b>1</b> : 599        | Gadgil Madhav <b>2</b> : 1447     |
| Antia D P <b>1</b> : 582                | Chandrasekhar S <b>1</b> : 143      | Ganapathi K <b>2</b> : 1348       |
| Appaji Rao N <b>2</b> : 1310            | Chatterjea J N <b>1</b> : 367       | Gangulee H C <b>2</b> : 885       |
| Arya A S <b>1</b> : 586                 | Chatterjee Asima <b>1</b> : 370     | Ganguli H C <b>2</b> : 1171       |
| Asana R D <b>2</b> : 851                | Chatterjee S D <b>1</b> : 146       | Ganguly A K <b>1</b> : 162        |
| Aswanatharayana U <b>1</b> : 720        | Chatterjee S N <b>2</b> : 1146      | Ganguly J <b>2</b> : 1352         |
| Auluck F C <b>1</b> : 122               | Chidambaram R <b>1</b> : 149        | Garde R J <b>1</b> : 622          |
| Awasthi D D <b>2</b> : 855              | Chopra S R K <b>2</b> : 1150        | George M V <b>1</b> : 397         |
| Bachhawat B K <b>2</b> : 1315           | Chopra V L <b>2</b> : 874           | Ghatak U R <b>1</b> : 399         |
| Balakrishnan Nair N <b>2</b> : 1025     | Choudhury Bishwajit <b>2</b> : 1432 | Ghatge V M <b>1</b> : 625         |
| Balbhadra Prasad <b>1</b> : 331         | Chowdhury A B <b>2</b> : 1153       | Ghildyal B P <b>2</b> : 1450      |
| Baliah V <b>1</b> : 333                 | Chowdhury Mihir <b>1</b> : 377      | Ghosh Asok <b>2</b> : 1046        |
| Bambah R P <b>1</b> : 3                 | Daniel R R <b>1</b> : 152           | Ghosh B N <b>1</b> : 402          |
| Banerjee D K <b>1</b> : 337             | Das B N <b>1</b> : 601              | Ghosh J K <b>1</b> : 14           |
| Banerjee Sachchidananda <b>2</b> : 1134 | Das J <b>1</b> : 604                | Ghosh N L <b>1</b> : 18           |
| Banerjee T <b>1</b> : 591               | Das P K <b>1</b> : 734              | Ghosh S K <b>1</b> : 743          |
| Barnabas John <b>2</b> : 1318           | Dasannacharya B A <b>1</b> : 155    | Ghosh S N <b>1</b> : 164          |
| Basu D <b>1</b> : 124                   | Das Gupta C R <b>2</b> : 1156       | Ghosh T K <b>2</b> : 1175         |
| Basu Sadhan <b>1</b> : 341              | Das Gupta N N <b>2</b> : 1337       | Gill K S <b>2</b> : 1453          |
| Bhaduri Amar Nath <b>2</b> : 1323       | Das Gupta S N <b>2</b> : 878        | Gill P S <b>1</b> : 168           |
| Bhakuni D S <b>1</b> : 345              | Dass C M S <b>2</b> : 1031          | Gopal Prasad <b>1</b> : 22        |
| Bhargava K S <b>2</b> : 859             | Dastur Darab K <b>2</b> : 1161      | Gopalan C <b>2</b> : 1178         |
| Bhargava P M <b>2</b> : 1325            | Datta Asoke G <b>2</b> : 1341       | Gopalan K <b>1</b> : 745          |
| Bhat J V <b>2</b> : 1140                | Datta Munshi J S <b>2</b> : 1034    | Gopala Rao R V <b>1</b> : 406     |
| Bhatia I S <b>2</b> : 1428              | De Rajat <b>2</b> : 1435            | Gopinath N <b>2</b> : 1181        |
| Bhatia M L <b>2</b> : 1028              | Deekshatulu B L <b>1</b> : 606      | Govil Girjesh <b>1</b> : 409      |
| Bhatia S B <b>1</b> : 724               | Deo M G <b>2</b> : 1164             | Govind Swarup <b>1</b> : 170      |
| Bhatt M V <b>1</b> : 347                | Deodikar G B <b>2</b> : 1438        | Govindachari T R <b>1</b> : 413   |
| Bhattacharyya A B <b>1</b> : 592        | Desai R D <b>1</b> : 380            | Govinda Rao N S <b>1</b> : 626    |
| Bhattacharyya J C <b>1</b> : 127        | Deshpande B G <b>1</b> : 738        | Gulatee B L <b>1</b> : 747        |
| Bhimasankaram V L S <b>1</b> : 726      | Desikachary T V <b>2</b> : 882      | Gupta A S <b>1</b> : 24           |
| Bilgrami K S <b>2</b> : 862             | Dey A K <b>1</b> : 741              | Gupta B M <b>2</b> : 1457         |
| Bir S S <b>2</b> : 864                  | Dhar M L <b>1</b> : 383             | Gupta H <b>1</b> : 26             |
| Biswas B B <b>2</b> : 1329              | Dhar M M <b>1</b> : 386             | Gupta P K <b>2</b> : 887          |



- Guraya S S 2 : 1049  
 Hari Narain 1 : 751  
 Hariharan P 1 : 171  
 Heilig Robert 2 : 1184  
 Huzurbazar S 1 : 29  
 Ishwar Prakash 2 : 1056  
 Iyengar P K 1 : 173  
 Iyer P V K 1 : 31  
 Jagannathan V 2 : 1355  
 Jagdish Shankar 1 : 419  
 Jai Krishna 1 : 628  
 Jain A C 1 : 422  
 Jain H K 2 : 1463  
 Jain M K 1 : 38  
 Jain P C 1 : 40  
 Jain S K 2 : 890  
 Jha S S 1 : 177  
 Jhingran V G 2 : 1058  
 Johri B M 2 : 893  
 Joshi B S 1 : 426  
 Joshi S K 1 : 182  
 Kanungo M S 2 : 1062  
 Kanwar J S 2 : 1466  
 Kapil R N 2 : 899  
 Kapil R S 1 : 430  
 Kapoor S S 1 : 184  
 Kapur J N 1 : 45  
 Kasturi T R 1 : 432  
 Katiyar S S 1 : 437  
 Kaw P K 1 : 187  
 Kedharnath S 2 : 1470  
 Kessar S V 1 : 442  
 Khan Abrar M 2 : 1475  
 Khatri C G 1 : 50  
 Khetrapal C L 1 : 444  
 Khoshoo T N 2 : 903  
 Khush Gurdev S 2 : 1479  
 Koteswaram P 1 : 755  
 Kothari L S 1 : 190  
 Krishna Murti C R 2 : 1357  
 Krishna P 1 : 193  
 Krishna Swarup 2 : 1065  
 Krishnan R 1 : 631  
 Krishnan R S 1 : 195  
 Krishnan V 1 : 447  
 Kullkarni A B 1 : 450  
 Kundu B C 2 : 908  
 Kushwaha R S 1 : 53  
 Lakhanpal R N 2 : 911  
 Lal D 1 : 199  
 Lal K B 2 : 1483  
 Laumas K R 2 : 1186  
 Luthra Usha K 2 : 1191  
 Madhavarao B S 1 : 202  
 Madhava Reddy G 2 : 1484  
 Maitra P K 2 : 1360  
 Majumdar C K 1 : 204  
 Majumdar R C 1 : 207  
 Malik C P 2 : 915  
 Mani A 1 : 758  
 Manna G K 2 : 1068  
 Manoharan P T 1 : 455  
 Mashekar R A 1 : 633  
 Mathews P M 1 : 209  
 Mathur K S 2 : 1195  
 Mehra P N 2 : 919  
 Mehra S R 1 : 637  
 Mehrotra K N 2 : 1073  
 Mehrotra R C 1 : 458  
 Menon M G K 1 : 212  
 Merh S S 1 : 760  
 Mishra R S 1 : 55  
 Misra R 2 : 926  
 Misra R C 1 : 762  
 Mitra A N 1 : 216  
 Mitra A P 1 : 220  
 Mitra H K 1 : 640  
 Mitra S K 1 : 57  
 Mitra S N 1 : 643  
 Modi V V 2 : 1489  
 Mohan Ram H Y 2 : 929  
 Mookerjee Sivatosh 2 : 1078  
 Mookherjee Asoke 1 : 765  
 Moudgal N R 2 : 1362  
 Mukherjee A S 2 : 1081  
 Mukherjee S K 2 : 1492  
 Mukunda N 1 : 62  
 Murty B R 2 : 1494  
 Murty Y S 2 : 935  
 Nag B R 1 : 224  
 Nagarajan K 1 : 461  
 Naha K 1 : 768  
 Nair K R 1 : 65  
 Nanjundayya C 1 : 226  
 Naqvi S M 1 : 772  
 Narasimha Roddam 1 : 645  
 Narasimham N A 1 : 232  
 Narasimhan R 1 : 649  
 Narayana Reddy G K 1 : 464  
 Narlikar Jayant V 1 : 235  
 Narlikar V V 1 : 67  
 Nasipuri D 1 : 468  
 Nath M C 2 : 1365  
 Nigam H L 1 : 472  
 Nijhawan B R 1 : 652  
 Nitya Anand 1 : 475  
 Notani N K 2 : 1371  
 Padmanaban G 2 : 1374  
 Pai M A 1 : 656  
 Pakrashi S C 1 : 479  
 Pal B P 2 : 1499  
 Pal R K 2 : 1198  
 Pande I C 1 : 775  
 Pandeya S C 2 : 940  
 Pandya S P 1 : 238  
 Pant D D 2 : 945  
 Paroda R S 2 : 1501  
 Parthasarathy K R 1 : 70  
 Parthasarathy N 2 : 1507  
 Parthasarathy R 1 : 74  
 Patel C C 1 : 481  
 Paul R C 1 : 485  
 Peters B 1 : 241  
 Pichamuthu C S 1 : 778  
 Pillai M K K 2 : 1085  
 Pisharoty P R 1 : 782  
 Prakasa Rao B L S 1 : 76  
 Prasad M R N 2 : 1088  
 Prem Narain 2 : 1510  
 Prihar S S 2 : 1514  
 Puri V 2 : 947  
 Qasim S Z 1 : 784  
 Radhakrishna B P 1 : 786  
 Radhakrishna Rao C 1 : 79  
 Raghavan S 1 : 82  
 Raghunathan M S 1 : 85  
 Raizada M B 2 : 953  
 Raja Gopal E S 1 : 244  
 Rajappa S 1 : 489  
 Rajaraman R 1 : 247  
 Rajasekaran G 1 : 249  
 Rajinder Kumar 1 : 658  
 Rakshit H 1 : 661  
 Ramachandra K 1 : 87  
 Ramachandran A 1 : 666  
 Ramachandran G N 2 : 1377  
 Ramachandran L K 2 : 1383  
 Ramachandrarao P 1 : 671  
 Ramachandra Row L 1 : 492  
 Rama Das V S 2 : 954  
 Ramakrishnan P S 2 : 958  
 Ramakrishnan T 2 : 1203  
 Ramakrishnan T V 1 : 253  
 Ramalingaswami V 2 : 1207  
 Ramamurthi B 2 : 1210  
 Ramana Murthy P V 1 : 255  
 Ramanan S 1 : 90  
 Ramanathan N 2 : 1388  
 Ramaniah M V 1 : 500  
 Ramanna Raja 1 : 257  
 Rama Rao A V 1 : 505  
 Rama Rao P 1 : 675  
 Ramaseshan S 1 : 260  
 Ramaswami L S 2 : 1092  
 Ramaswamy G S 1 : 679  
 Ramiah K 2 : 1518  
 Ranadive Kamal J 2 : 1212  
 Randhawa M S 2 : 1522  
 Randhawa N S 2 : 1524  
 Ranganathan S 1 : 510, 683  
 Rangaswami S 1 : 514  
 Rao C N R 1 : 518  
 Rao K J 1 : 523  
 Rao N G P 2 : 1528  
 Rao P S 1 : 527  
 Rao P V S 1 : 686  
 Rao S R V 2 : 1096

- Rao U R 1 : 264  
 Rao V S R 2 : 1392  
 Rastogi R G 1 : 789  
 Rastogi R P 1 : 531  
 Ray A P 2 : 1216  
 Raychaudhuri, Satya P 2 : 1533  
 Raychaudhuri, Syama P 2 : 1536  
 Reddy C R R M 2 : 1221  
 Rohatgi-Mukherjee K K 1 : 535  
 Roonwal M L 2 : 1098  
 Roy Amalendu 1 : 791  
 Roy B B 2 : 1226  
 Roy R P 2 : 961  
 Roy Supriya 1 : 794  
 Rudraiah N 1 : 93  
 Sadasivan T S 2 : 965  
 Sadhu D P 2 : 1540  
 Saha A K 1 : 797 2 : 1232  
 Saha N K 1 : 268  
 Saksena S B 2 : 969  
 Salim Ali 2 : 1101  
 Sandhu S S 1 : 539  
 Santappa M 1 : 542  
 Sanyal G S 1 : 690  
 Sarkar J K 2 : 1235  
 Sarma Y S R K 2 : 972  
 Sasisekharan V 2 : 1397  
 Sastri M V C 1 : 547  
 Sastry P S 2 : 1401  
 Sathyanesan A G 2 : 1104  
 Satya Prakash 1 : 272  
 Seal S C 2 : 1239  
 Sen A B 2 : 1245  
 Sen H G 2 : 1247  
 Sen P K 2 : 1251  
 Sen S K 1 : 800  
 Sen S P 2 : 976  
 Sen Saurindranath 1 : 802  
 Sengupta P 1 : 551  
 Sengupta S 1 : 805  
 Seshadri C S 1 : 96  
 Shah J J 2 : 979  
 Shah S M 1 : 99  
 Shah V C 2 : 1107  
 Sharma A K 2 : 982  
 Sharma Archana 2 : 986  
 Sharma G P 2 : 1109  
 Sharma K N 2 : 1255  
 Sharma M M 1 : 692  
 Sharma V P 2 : 1111  
 Shastry S V S 2 : 1542  
 Shorey T N 1 : 102  
 Shrikhande S S 1 : 103  
 Siddiqui M Raziuddin 1 : 106  
 Siddiquie H N 1 : 808  
 Singh B N 2 : 990  
 Singh D V 1 : 695  
 Singh J S 2 : 994  
 Singh M P 1 : 108  
 Sinha A K P 2 : 1257  
 Sinha A P B 1 : 554  
 Sinha K P 1 : 276  
 Sinha M S 1 : 280  
 Sinha S K 2 : 997  
 SivaRaman C 2 : 1404  
 Sodha M S 1 : 282  
 Sohi H S 2 : 1000  
 Somayajulu B L K 1 : 812  
 Sreekantan B V 1 : 285  
 Sreenivasan A 2 : 1407  
 Srinivasan M S 1 : 815  
 Srinivasan S 2 : 1409  
 Sriramachari S 2 : 1261  
 Srivastava Har Dayal 2 : 1114  
 Srivastava P N 2 : 1118  
 Srivastava U S 2 : 1120  
 Subba Rao N S 2 : 1545  
 Sukhatme P V 2 : 1267  
 Sukh Dev 1 : 557  
 Sukheswala R N 1 : 818  
 Sundaram C V 1 : 697  
 Sundaram K 2 : 1272  
 Surange K R 2 : 1004  
 Swaminathan M 2 : 1276  
 Swaminathan M S 2 : 1548  
 Swaminathan S 1 : 564  
 Talwar G P 2 : 1279  
 Tandon P N 2 : 1286  
 Tandon R N 2 : 1007  
 Taqui Khan M M 1 : 568  
 Thirumalachar M J 2 : 1009  
 Thiruvenkatachar V R 1 : 111  
 Thomas, Joseph 2 : 1412  
 Thosar B V 1 : 290  
 Toshniwal G R 1 : 701  
 Trehan S K 1 : 115  
 Trivedi B S 2 : 1014  
 Udgaonkar B M 1 : 293  
 Udupa H V K 1 : 702  
 Uppal H L 1 : 707  
 Vaidyanath L R 1 : 711  
 Vaidyanathan C S 2 : 1416  
 Valdiya K S 1 : 825  
 Valiathan M S 2 : 1291  
 Varadachari V V R 1 : 828  
 Varma R K 1 : 297  
 Venkataraman G 1 : 302  
 Venkataraman G S 2 : 1017  
 Venkateswarlu Putcha 1 : 305  
 Verma Ajit Ram 1 : 312  
 Vig O P 1 : 570  
 Vijayaraghavan R 1 : 315  
 Virendra Singh 1 : 317  
 Viswanathan M N 1 : 831  
 Wadia N H 2 : 1293  
 Wahi P N 2 : 1296  
 West W D 1 : 834  
 Yadava J N S 2 : 1552











### **Some Important Recent Publications of the Academy**

1. Science in India; 50 Years of the Academy
2. Fellows of the Indian National Science Academy (Past and Present) —1935-84
3. INSA Medal Lectures (Volumes I & II)—1950-83
4. Biographical Memoirs of the Fellows of the Academy (Volumes 1-10)

### **Periodicals**

5. Indian Journal of Pure and Applied Mathematics
6. Proceedings of the Indian National Science Academy  
Part A (Physical Sciences)  
Part B (Biological Sciences)
7. Indian Journal of History of Science
- \*8. International Science News & INSA Delegation Reports
- \*9. INSA News

\*Limited circulation.



